BIOCATALYTIC POLYESTER SYNTHESIS IN SUPERCRITICAL CARBON DIOXIDE, IONIC LIQUIDS AND ORGANIC SOLVENTS

by

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B.S. in Ch.E., University of Pittsburgh, 1997

Submitted to the Graduate Faculty of

the School of Engineering in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2002

UNIVERSITY OF PITTSBURGH

SCHOOL OF ENGINEERING

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ABSTRACT

BIOCATALYTIC POLYESTER SYNTHESIS IN SUPERCRITICAL CARBON DIOXIDE, IONIC LIQUIDS AND ORGANIC SOLVENTS

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The use of enzymes as catalysts in chemical reactions has been an intense area of research for many years. Biocatalysis allows for reactions which would normally require high temperatures and pressures, as well as corrosive acid catalysts, to be carried out at ambient conditions while being highly selective. By pairing enzymes with environmentally benign solvents, such as supercritical carbon dioxide and ionic liquids, the detrimental impact of traditional chemical processes on the environment could be significantly reduced.

Polyester synthesis in supercritical carbon dioxide would allow for the rational control of polymer molecular weight and dispersity due to the solvent tunability of supercritical fluids. In order to overcome poor monomer solubility in carbon dioxide, fluorinated monomers were substituted. Thus, fluorinated polyester synthesis was performed from activated diesters and fluorinated diols and the factors that would limit chain extension were assessed. Weight average molecular weights of up to 8094 Da were achieved. The phase behavior of each monomer, as well as the polyester product, was also studied. Additionally, hydroxylated monomers were incorporated into the synthesis

in order to produce polyesters that had fluorinated, hydrogenated, and hydroxylated segments.

In the Novozym® 435-catalyzed reaction between divinyl adipate and 1,4-butanediol, carbon dioxide was also employed as a viscosity reducing agent in order to alleviate some of the mass transfer limitations that occur during the polymerization. The presence of CO_2 did enhance polymer molecular weight but only at reaction times of 1 hour or less. At reaction times longer than 1 hour, the presence of CO_2 hindered the achievable molecular weight.

Ionic liquids are another environmentally friendly solvent in which enzymatic reactions can be carried out. The enzymes Novozym® 435 and porcine pancreatic lipase were found to show enhanced stability in an ionic liquid when compared to a traditional organic solvent. The biocatalytic polymerization between divinyl adipate and 1,4-butanediol was carried out in this medium but polymer molecular weight was limited due to the precipitation of the polymer from the ionic liquid.

DESCRIPTORS

Biocatalysis
Green
Ionic Liquid
Molecular Weight
Organic Solvent
Polyester
Pore
Supercritical Fluid
Fluorinated Polyester
Hydroxyl Group
Lipase
Nonaqueous Enzymology
Phase Behavior
Polytransesterification
Stability
Viscosity

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Alan Russell, for his support and guidance during my time here at the University of Pittsburgh, not only in graduate school but in undergraduate as well. He has been and always will be someone that I look up to with great respect and admiration. I would also like to thank my co-advisor, Dr. Eric Beckman, for his time, advice and knowledge.

I am especially grateful to Dr. Robert Enick for his input on certain aspects of this research project and for being a great source of encouragement. I would also like to express my gratitude to Dr. Michael Sacks for his participation on my thesis committee.

Thank you to all the members of the Russell lab, past and present. I have made many great friends there these past few years and look forward to continuing these friendships for years to come.

Financial support from the National Science Foundation and the Environmental Protection Agency is gratefully appreciated.

I thank my parents, Roger and Jane Mesiano, for their love and support these past 26 years. All that I am today is because of them and I could not imagine having two better people as my parents. I am proud to call them Mom and Dad.

I would also like to thank my grandfather, Salvatore Mesiano, for his unconditional love. He is truly an inspiration.

Lastly, I would like to express my deepest thanks to my husband, Gary Jesionowski. I am eternally grateful for his love, support, encouragement, and

enthusiasm. He truly is my best friend in the world and I will always be indebted to him for the joy and happiness he has brought into my life.

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1.0 INTRODUCTION

1.1 Nonaqueous Enzymology

The use of enzymes in nonaqueous media, first inspired by the work of Klibanov, has been a growing area of research for the past two decades.(1-5)[*] Although the natural environment for an enzyme is water, enzymes have been shown to exhibit unique traits in organic solvents. Table 1 lists several advantages of employing enzymes in organic media.(1,2)

Table 1 Advantages of Employing Enzymes in Organic Solvents

Solubility of hydrophobic compounds is increased
Immobilization is not necessary because enzymes are insoluble in organic solvents. This also allows for the ease of enzyme recovery and reuse
Undesirable side reactions, such as hydrolysis of acid anhydrides and polymerization of quinones, are suppressed since water is needed for these reactions to occur
Thermodynamic equilibria is shifted in favor of synthesis of compounds such as esters and peptides
Elimination of microbial contamination
Enhanced thermostability
Alteration of substrate specificity
Ease of product recovery

However, a major drawback to the use of organic solvents is that they are not environmentally benign. Therefore, the following challenge has been proposed to

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[*] Parenthetical references placed superior to the line of text refer to the bibliography

researchers: how to convert the American chemical process industry into one that is more environmentally friendly, yet economically viable. The replacement of conventional chemical catalysts with enzymes, nature's catalysts, is a vital step towards this goal due to the fact that enzymes require mild reaction temperatures to function, thus eliminating the need for corrosive acid catalysts. Another advantage of enzymes lies in their high selectivity. Enzymes possess three different types of selectivity: regioselectivity (the ability to distinguish between various copies of the same functional group), chemoselectivity (the ability to distinguish between different functional groups on a compound), and enantioselectivity (the ability to distinguish between enantiomers).

1.1.1 Supercritical Fluids

Another step towards a harmonious relationship between the chemical industry and the environment could be accomplished by replacing traditional organic solvents with supercritical fluids as the reaction medium. Supercritical fluids are materials above their critical temperature, T_c, and critical pressure, P_c. Their properties lie between those of liquids and gases. Supercritical carbon dioxide is a particularly attractive solvent because it is nontoxic, inexpensive and environmentally benign.(6) The physical properties of supercritical fluids can be manipulated by simply changing the pressure or temperature.(7-11) Small changes in pressure can in turn lead to dramatic changes in density, thereby altering all density-dependent properties, such as dielectric constant, solubility parameter and partition coefficient.(12,13) This is particularly advantageous in enzymatic reactions since research has clearly demonstrated that the activity of enzymes is dependent on solvent properties.(2,3) Additionally, it has been shown that the activity(14) and selectivity(15) of enzymes can be modulated in supercritical fluids through changes in pressure or temperature. A further benefit of using supercritical fluids as the reaction medium is that their gaslike diffusivities and low viscosities enhance mass transfer rates of reactants to the active sites on enzymes that are dispersed in the solvent. Reactions that are limited by rates of diffusion, rather than intrinsic kinetics, would proceed faster in supercritical fluids.

Supercritical carbon dioxide is not only attractive for use as a reaction medium, but it is also widely used as a plasticizing agent. By adding carbon dioxide to polymer solutions, the viscosity of the solution may be lowered.(16-18) This process can be attributed to two mechanisms. First, the carbon dioxide can absorb between the polymer chains, thus reducing chain entanglement and increasing the free volume. Second, carbon dioxide is thought to act as a molecular lubricant. When these two effects are combined, the result is a reduction in the viscosity of the polymer solution.(17) Thus, if the achievable molecular weight of a polymer is hindered due to the fact that the viscosity of the polymer solution is too high during polymerization, addition of carbon dioxide may be able to alleviate this effect.

1.1.2 Ionic Liquids

Ionic liquids are room temperature liquids that are comprised entirely of ions. They remain liquid over a broad temperature range, are thermally stable and can dissolve a variety of compounds. More importantly, they have no measurable vapor pressure, thus eliminating the emission of toxic vapors to the environment.(19-21) Ionic liquids are typically comprised of small, negatively charged ions (anions) and large, bulky, positively charged ions (cations). The immense number of possible combinations of anions and cations gives scientists the ability to custom synthesize an ionic liquid that is compatible with the needs of their reaction systems. Additionally, catalysts and products can be extracted and recovered, allowing for recycling and reuse of the ionic liquid.

A problem encountered when using hydrophilic organic solvents as a reaction medium is that the water essential for the enzyme to maintain activity is partitioned away.(22) Moisture stable ionic liquids may be able to alleviate this problem by providing a stabilized ionic environment, while possessing strong solvating power. One hypothesis is that the ionic liquid acts as a matrix, thus providing the enzyme that is enclosed within it a more suitable conformation for retaining its activity.(23) Additionally, enzymes have been shown to exhibit improved enantioselectivity in ionic liquids.(24)

1.2 Polyesters

A major component of the nation's chemical industry is the production of polymers. Polyesters, in particular, are of great commercial importance since they are used in various applications such as polyurethane intermediates, alkyd resins, molding plastics and thermosets. Polyesters are polymers in which the repeat unit contains an ester linkage. They can be synthesized via three routes: condensation polymerization of diacids with diols, self-condensation of a hydroxycarboxylic acid, or ring-opening polymerization of lactones. While conventional chemical synthesis of these materials requires temperatures greater than 200ºC, it has been shown that polyesters can be synthesized enzymatically at 50ºC. Additionally, previous studies have demonstrated that polyester molecular weight and polydispersity can be controlled by using supercritical fluids as the reaction medium.(25)

While supercritical carbon dioxide would be an attractive solvent for the enzymatic synthesis of polymers, many of the reactants used in polymer synthesis are poorly soluble in carbon dioxide since it is a relatively nonpolar compound.(26) In the past, modifiers, namely alcohols, were used to overcome this obstacle.(27-31) However, this technique increases the critical temperature of the solvent, thus increasing the temperature and pressure of the reaction and, more importantly, adds an element of toxicity to the process. Therefore, research has focused on investigating functional groups that could enhance the solubility of compounds in carbon dioxide. Because both perfluorinated alkanes and carbon dioxide exhibit significantly low polarizability/dipolarity parameters (the ability of a solvent to stabilize a charge or dipole by its dielectric effect), several investigators have suggested that fluorination of a compound could increase its solubility in carbon dioxide.(32-37)

1.2.1 Fluorinated Polyesters

The inclusion of fluorine groups into polymers, more specifically, polyesters,(38-41) has enabled the synthesis of materials with improved chemical resistance, low water absorption, low coefficients of friction, and low surface energies. In addition, fluorinated hydroxyl-terminated polyesters have been used as polyurethane prepolymers(42) and it has also been found that the surface properties of fluorinated polyesters make them attractive for use as biomaterials.(41)

The synthesis of fluorinated polyesters is difficult for a variety of reasons. For example, due to their instability, fluorinated polyesters that have been prepared from perfluorinated acids have not been used as polyurethane prepolymers.(43,44) Other studies have used hydrogenated aliphatic diacids with fluorinated diols(39,40) as monomers for the polymerizations. However, it has been hypothesized that the inductive effect of the fluorine atoms decreases the reactivity of the diol.(45) This is because the high electronegativity of the fluorine atoms attracts the shared electrons. Because a negative charge is more stable when it is located on a more electronegative atom, the stability of the molecule is greatly increased. Therefore, the fluorinated form of the diol will be less reactive than the unfluorinated form. In order to reduce this effect, fluorinated diols that contain an additional methylene spacer between the hydroxyl functionality and the fluorine atoms can increase diol reactivity.(40) Additionally, other studies have used perfluoropolyethers as a route for synthesizing these materials.(46) However, the harsh reaction conditions required by some of these methods, such as high temperatures and acid chlorides are not conducive to the synthesis of these relatively delicate polymers.

1.2.2 Fluorinated-Hydroxylated Polyesters

Using enzymes to synthesize polymers provides the added benefit of specificity. This is particularly advantageous when trying to acylate hydroxyl compounds. Enzymes such as lipase from *Candida antarctica*,(47-49) porcine pancreatic lipase,(50-52) and subtilisin(53) have been successful at accomplishing this task. More importantly, lipase from *Candida antarctica*(47,54) has demonstrated selectivity towards primary hydroxyl groups, thus leaving the secondary hydroxyl groups unacylated.(47) It has recently been shown that linear hydroxyesters can be synthesized using enzymes.(55) Polyesters with pendant hydroxyl groups can increase the hydrophilicity of the polymer and therefore increase the solubility of the polyester in water. Water-soluble polyesters have a wide variety of uses such as in coatings, inks, textiles, and are particularly applicable in the field of biomaterials. Additionally, because enzymes are highly stereoselective, reactions that generate polyesters with pendant functional groups can also be investigated for enantioselectivity. If a given reaction is enantioselective, this would yield a functionalized, optically active polymer.

The synthesis of a copolymer containing fluorinated and hydroxylated segments would produce a material whose backbone is highly hydrophobic in given segments, yet the material would have regions of hydrophilicity as well, due to the pendant hydroxyl groups. A polymer such as this is likely to have interesting properties and could be highly applicable as a compatibilizer for immiscible polymer blends. While the synthesis of these copolymers can be challenging, compatibilizers are an effective way to improve phase dispersion and interfacial adhesion and to stabilize phase morphology.(56-58)

2.0 BACKGROUND AND LITERATURE REVIEW

2.1 Enzymatic Synthesis of Polyesters

The biocatalytic synthesis of polyesters dates back to the early 1980's. Okumura et al.(59) attempted to synthesize polyesters from various diacids and diols. The diacids ranged from six to fourteen carbons and the diols were either 1,2-ethanediol or 1,3-propanediol. The enzyme used for these studies was lipase from *Aspergillus niger* NRRL 337. However, the longest chain produced was only that of heptamer. The authors concluded that the key factor in limiting production of higher molecular weight materials was due to solubility problems of the product in the reaction mixture.

Klibanov and coworkers(60) attempted to synthesize optically active polyesters via the enzyme-catalyzed transesterification of diesters with diols. The enzymes used were lipases from *Aspergillus niger* and porcine pancreas. While optical activity was achieved, the molecular weights of the polymers were very low. The average degree of polymerization was only one to three repeat units and the average molecular weight of the materials was less than 1000 Daltons.

Wallace and Morrow(61) continued in this line of work by carrying out the enantioselective polymerization of bis (2,2,2-trichloroethyl) *trans*-3,4-epoxyadipate with 1,4-butanediol using lipase from porcine pancreas in anhydrous ethyl ether at ambient temperature. By using the trichloroethyl ester, this made the leaving groups much more active. Therefore, the average degree of polymerization grew to twenty-five repeat units and the weight average molecular weight was 7900 Daltons. The authors published a follow-up paper(62) in which polyesters were synthesized from bis (2,2,2-trichloroethyl) alkanedioates and diols using lipase from porcine pancreas in solvents such as ether, tetrahydrofuran, and mixtures of methylene chloride with hexane and ether. Gel permeation chromatography results indicated that polymers with weight average molecular weights as high as 14,900 Daltons were achieved.

Brazwell et al.(63) addressed the issue of limitation of polymer molecular weight due to hydrolysis and the equilibrium position of the reaction. The authors concluded that hydrolysis of the activated ester groups was occurring via the water that was introduced to the reaction system from the enzyme. Further, the polymerization appeared to reach equilibrium even though bis (2,2,2-trifluoroethyl) glutarate was used as one of the reactants, which thus produced 2,2,2-trifluoroethanol as the leaving group. The authors also found that the trifluoroethanol accelerated the release of the water bound to the enzyme. It was concluded that these issues could be overcome by extensively drying the enzyme, using a high-boiling solvent, or by placing the reaction mixture under vacuum.

Dordick and coworkers(64,65) have performed extensive research on synthesizing polyesters with sugars incorporated into the backbones of the polymers. After a screen of over sixty commercially available enzymes, it was found that Proleather, an Amano enzyme from *Bacillus* protease, was able to polymerize sucrose with adipic acid derivatives in anhydrous pyridine. The resulting polymer had up to 100 sucrose units incorporated into it. The authors were also able to incorporate raffinose, lactose, and fructose into the polyester backbone as well. More recently, Dordick and coworkers(66,67) have been able to enzymatically synthesize sucrose-containing monomers and then polymerize them with ethylene-diamine, ethylene glycol, and various other diols (both aliphatic and aromatic) to produce sucrose-containing polymers.

Chaudhary et al.(68) were able to synthesize high molecular weight polyesters in a solvent-free environment at 50°C using divinyl adipate, an activated diester, and 1,4-butanediol. The reaction was catalyzed by Novozym®435, a lipase from *Candida antarctica* that is immobilized onto a macroporous acrylic resin. Polyesters with weight average molecular weights in excess of 20,000 Daltons were achieved with relatively small concentrations of enzyme. The authors also found that the enzyme was able to be recycled five times and still produced polyesters with high molecular weights.

Chaudhary et al.(69) also demonstrated that Novozym®435 has a higher specificity for transesterification of ester-terminated oligomers during the initial phase of the reaction. However, at different stages of the polymerization, there appears to be a competition between hydrolysis and transesterification. Hydrolysis can limit polymer growth and terminate the polymer with acid end groups. The authors also found that the functionality of the product is influenced by a variety of factors, including enzyme concentration, water content, enzyme specificity, and the stoichiometric ratio of the reactants when the polymerization is begun.

Binns et al.(70) studied the Novozym®435-catalyzed reaction between adipic acid and 1,4-butanediol in a solvent-free environment, as well as in toluene. The authors suggest that the specificity of the enzyme changes when it is in an organic solvent as opposed to a solvent-free system. It was hypothesized that at the start of the reaction, an intermediate, formed between the adipic acid and 1,4-butanediol, becomes acylated with the enzyme. The polymer chain then propagates by reacting with a hydroxy-terminated species. The enzyme is then released and allowed to further react, eventually forming long polymer chains.

Kitagawa and Tokiwa(71) were able to synthesize an ester from divinyl sebacate and glucose using a variety of lipases. The reaction took place in pyridine at 30°C for seven days. Of the five lipases tested, the one which gave the highest conversion was lipase from *Alcaligenes sp*. However, lipases from *Pseudomonas sp*. and *Candida antarctica* also gave relatively high conversions. The sugar ester product was then polymerized in DMF with 2,2′-azobis (isobutyronitrile) to give a branched polymer, poly (6-*O*-vinylsebacyl-D-glucose) and had a weight average molecular weight of 16,400 Daltons.

Kikuchi et al.(72) produced optically active polyesters through the lipase-catalyzed enantioselective copolymerization of substituted lactones. *Candida antarctica* lipase was used as the catalyst and the authors were able to show that the (S) isomer of racemic β-butyrolactone was preferentially reacted to give a product with an enantiomeric excess of 69%.

Warwel et al.(73) biocatalytically synthesized polyesters through lipase-catalyzed polycondensation of long-chain unsaturated and epoxidized α,ω-dicarboxylic acid methyl esters with diols. When 1,3-propanediol was used as a substrate, molecular weights ranged from 1950-3300 g/mol. However, when 1,4-butanediol was used instead, polymer molecular weight increased to a range of 7900-11,600 g/mol. In both instances, Novozym® 435 was used as the catalyst.

In a new approach, Kim and Dordick(74) applied a combinatorial strategy for enzymatic polyester synthesis. A library of polymers was synthesized using AA-BB polycondensations of various acyl donors (straight-chain diesters) and acceptors (aliphatic/aromatic diols, nucleic acids, carbohydrates, and a natural steroid diol) in 96 deep-well plates. Lipase from *Candida antarctica* was used as the catalyst in all of the reactions and molecular weights of up to 20,000 Da were achieved.

2.2 Supercritical Biocatalysis

Some of the earliest work with supercritical carbon dioxide was performed by Randolph et al.(75) The enzyme alkaline phosphatase was found to be active in a batch reaction system that employed supercritical carbon dioxide as the reaction medium. The enzyme catalyzed the reaction of disodium *p*-nitrophenyl phosphate in which *p*-nitrophenol was produced, but was limited by the solubility of disodium *p*-nitrophenyl phosphate in supercritical carbon dioxide. However, the enzyme was shown to be active after exposure to the solvent for twenty-four hours.

The enzymes employed in most of the work involving supercritical fluids, and more specifically, carbon dioxide have been lipases. Extensive work has been carried out by various research groups on a wide variety of reactions. Nakamura et al.(76-78) studied the acidolysis of triolein with stearic acid in supercritical carbon dioxide in both a batch and continuous reactor. Four lipases were used, three of which were immobilized. The enzymes were found to be stable in the reaction environment and the combination of a high substrate concentration, low water content, and short residence time resulted in better productivity from the reaction.

Marty et al.(79,80) performed an extensive study on the lipase-catalyzed esterification of oleic acid by ethanol. It was found that the lipase from *Mucor miehei*, commercially known as Lipozyme, required approximately 10% (w/w) water content in order to achieve maximum activity. When water concentration exceeded 200mM, enzyme activity was reduced and continued to decrease as more water was added to the system.

Dumont and Barth(81) performed the esterification of myristic acid using an immobilized lipase from *Mucor miehei* in both *n*-hexane and supercritical carbon dioxide. Although the reaction exhibited a higher maximum velocity in the carbon dioxide, the myristic acid was found to be more soluble in *n*-hexane, thus calling into question whether or not carbon dioxide is a viable solvent alternative due to the fact that many substrates are not soluble in it.

The issue of substrate solubility in supercritical carbon dioxide was studied by Yoon et al.(82) The transesterification reaction between triolein and either behenic acid or its ethyl ester was carried out with an immobilized lipase. The ethyl ester of behenic acid was found to be approximately 1000 times more soluble in supercritical carbon dioxide than that of behenic acid. Consequently, the rate of the reaction in which the ethyl ester was used was higher than the one in which the acid was used.

Performing enzymatic reactions in supercritical carbon dioxide can also be used to produce optical isomers via chiral synthesis or resolution of a racemic mixture.

Ikushima et al.$^{(83)}$ studied the transesterification of (±) citronellol with oleic acid catalyzed by the lipase *Candida cylindracea*. As the pressure was increased, the rate of the reaction also increased. This effect was especially noticeable at the critical point. Also, the optical purity of the product was found to be sensitive to pressure. Around the critical point, the *S* ester was stereoselectively formed. However, at higher pressures, the optical purity was much less. Endo et al.$^{(84)}$ also produced chiral esters from secondary alcohols and short-chain fatty acids using two immobilized lipases in supercritical carbon dioxide. The reactions catalyzed by lipase OF produced both the *R* and *S* forms of the ester, while Lipozyme-catalyzed reactions yielded only the *R* form of the ester.

Barreiros et al.$^{(85)}$ studied the activity of Novozym®435 in supercritical carbon dioxide, supercritical ethane, and compressed propane. The enzyme was used to catalyze the transesterification of butyl acetate by *n*-hexanol. It was found that at 35°C and 10,000 kPa, the activity of Novozym®435 was similar in supercritical ethane and compressed propane, but approximately one order of magnitude lower in carbon dioxide. However, the reaction rate did increase with temperature in supercritical carbon dioxide, whereas it did not increase at all in compressed propane and only slightly in supercritical ethane.

Liaw et al.$^{(86)}$ investigated the continuous synthesis of phenylethyl acetate by the lipase-catalyzed esterification of phenylethanol with acetic acid in supercritical carbon dioxide. By varying parameters such as water content, reaction temperature and pressure, substrate concentration, and gas flow rate, optimum operating conditions were found for the reaction. When water content was kept below 3% (w/w), the conversion rate was approximately 70%. However, the enzyme was irreversibly inactivated when water content was greater than 8% (w/w).

Kamat et al.(87) studied the transesterification of methylmethacrylate with 2-ethylhexanol in a variety of supercritical fluids. For this particular reaction system, supercritical carbon dioxide was found to be a very poor solvent when compared to other supercritical fluids. This was not surprising due to the fact that when the alcoholysis of methylmethacrylate was carried out in hexane, the presence of carbon dioxide inhibited the activity of the enzyme. It was proposed that carbon dioxide formed reversible carbamate complexes with the free amine groups on the surface of the enzyme. Direct evidence for carbamate formation was later shown through laser desorption mass spectroscopy (LD-MS).(88)

Chaudhary et al.(25) studied the lipase-catalyzed polytransesterification between bis (2,2,2-trichloroethyl) adipate and 1,4-butanediol in supercritical fluoroform at 50°C. The authors found that pressure could be used to control the molecular weight and polydispersity of the resulting polymer. Increasing the pressure increases the solvent solubility parameter. As a result, the molecular weight of the polymer that can be solubilized by the fluid and extracted from it increases with increasing pressure. Additionally, because the precipitated polymer has a low rate for chain extension, its polydispersity will remain low. The precipitation of the product was also found to drive the equilibrium of the reaction in the forward direction. When the same reaction was performed in anhydrous ether, it was found that the polymerization was equilibrium limited.

Fontes et al.(89) studied crystals of subtilisin Carlsberg in supercritical carbon dioxide and ethane. The enzyme was used to catalyze the transesterification of *N*-acetyl-*L*- phenylalanine ethyl ester by 1-propanol at 40°C and 100 bar. It was found that the activity of the enzyme was 2 to 10 fold greater in supercritical ethane than in *n*-hexane, as long as the CLECs were dried by propanol washing. Air-dried CLECs and lyophilized enzymes resulted in lower catalytic activities.

Osanai et al. (90) applied supercritical carbon dioxide to biocatalysis in a different manner. After performing the lipase-catalyzed ring-opening polymerization of β-butyrolactone in bulk, the structure of the polymer product was analyzed via supercritical carbon dioxide chromatography. This technique was able to confirm that the polymerization did occur and yielded cyclic, hydroxy- and crotonate-terminated poly(3-hydroxyalkanoate).

2.3 The Solubility of Fluorinated Compounds in Supercritical Carbon Dioxide

In the past decade, there has been an abundant amount of research that has investigated the solubility of various fluorinated compounds in supercritical carbon dioxide. Iezzi et al.(91) studied "gel" formations in mixtures of dense carbon dioxide and semifluorinated alkanes. This occurred when an equilibria between three phases, solid-liquid-vapor, was established. The liquid carbon dioxide became intertwined with the solid phase, which had formed microfibers. Thus, the gel was a mixture of the solid and liquid phases. The authors were also able to establish that fluorinating an alkane enhanced its solubility in carbon dioxide. This was determined by observing that a carbon dioxide/perfluorohexane system had a much higher degree of miscibility than a carbon dioxide/hexane system at 41.5°C and 81°C.

Laintz et al.(37) studied the separation of metal complexes in supercritical carbon dioxide and found that by fluorinating the diethyldithiocarbamate ligand, the solubility of metal chelates in carbon dioxide was greatly improved by several orders of magnitude. The authors determined the solubilities and behavior of these compounds spectroscopically by using UV-VIS and FT-IR.

Hoefling et al.(92) found that by incorporating a fluoroether functional group, hexafluoropropylene oxide, as the repeating unit in a perfluorinated alkylpolyether, the most carbon dioxide-soluble polymer to date was observed. The authors also found that by adding perfluoroalkylpolyethers as hydrophobic tails to surfactants, the solubility of the surfactant in carbon dioxide was also greatly enhanced.

Newman et al.(93) continued this line of research by studying the phase behavior of fluoroether-functional amphiphiles in supercritical carbon dioxide. It was concluded that there are several factors in competition with one another which determine the overall solubility of a compound in carbon dioxide. Increasing the chain length of the compound increases its molecular weight and subsequently forces the cloud point curve to a higher pressure. Also, increasing the polarity of the polar head group causes the cloud point curve to move to higher pressures. However, the addition of fluorine groups into the structure works to counterbalance these effects by moving the cloud point curve to lower pressures.

Harrison et al.(94) found that a hybrid fluorocarbon/hydrocarbon surfactant, $C_7F_{15}CH(OSO_3^{-}\text{-}Na^{+})C_7H_{15}$, formed a water-in-carbon dioxide microemulsion which solubilized large amounts of water. Using 1.9 wt% surfactant, 2 wt% water was found to be soluble in carbon dioxide. This is ten times the amount that is soluble in pure carbon dioxide. The authors also formulated a set of requirements for designing surfactants that would form microemulsions in carbon dioxide: 1) the tails of the surfactant should be CO_2-philic. In other words, have a low solubility parameter or low polarizability per volume; 2) Either two tails or a branched tail should be used in order to bend the interface around the water; 3) The head group should hydrogen bond with the water.

Tuminello et al.(95) studied the solubility of a copolymer of tetrafluoroethylene (TFE) and hexafluoropropylene (HFP) in supercritical carbon dioxide and compared the results to theoretical predictions made from phase diagrams of carbon dioxide with pure tetrafluoroethylene and its copolymers. The authors found that the experimental results agreed reasonably well with the predicted ones. Experimentally, the copolymer dissolved at 165°C and 90MPa. The theoretical results were calculated to be 170°C and 100MPa. The authors concluded that supercritical carbon dioxide is a reasonable solvent for dissolving perfluoropolymers.

Mertdogan et al.(96) compared the solvating power of supercritical carbon dioxide with other supercritical halogenated solvents by studying the solubility of poly(tetrafluoroethylene-*co*-19 mol% hexafluoropropylene) in each of these solvents. It was found that the halogenated solvents dissolved the polymer at much lower temperatures and pressures than carbon dioxide did. Temperatures greater than 185°C were needed to dissolve the copolymer. This was attributed to the fact that when compared to the halogenated solvents, carbon dioxide is a much more polar molecule due to its quadrupole moment, which is approximately three times as great as the dipole moment of one of the other solvents studied, $CClF_3$.

Beckman and Yazdi(97) synthesized chelating agents comprised of fluoroether picolyl amine, fluoroether bis(picolyl amine), fluoroether dithiocarbamate, and fluoroether dithiol. Several different types of these chelating agents were made in which the head group was separated from the fluorinated tail by various chain lengths of alkyl spacers. The authors found that the extraction efficiency for lead increases as the number of alkyl spacers increases for up to two carbons in length. After this length, increasing the spacer length had no significant effect on the extraction efficiency.

Enick et al.(98) studied the phase behavior of mixtures of fluoroether polyurethane precursors and liquid carbon dioxide. The authors found that a hydroxyl-terminated fluorinated polyether was miscible with carbon dioxide at pressures greater than 39.8 MPa and that a difunctional, isocyanate-terminated fluorinated polyether was miscible at pressures greater than 25.2 MPa. Polymerizations of these materials were also carried out in carbon dioxide at 42.2 MPa for 24 hours. The resulting cross-linked polyurethane was found to be completely soluble in carbon dioxide when the initial precursor was at a concentration of 4 wt% or less.

Dardin et al.(99) used high-pressure, high-resolution NMR in order to study solvent effects and solute-solvent interactions of *n*-hexane, perfluoro-*n*-hexane, and 1,1-dihydroperfluorooctylpropionate dissolved in supercritical and liquid carbon dioxide.

The results indicated that van der Waals interactions are present between the carbon dioxide and the fluorinated compounds, which constituted the first spectroscopic evidence of specific intermolecular interactions between carbon dioxide and fluorinated substances. It was also found that these interactions can vary by changing the pressure of carbon dioxide.

Campestrini et al. (100) synthesized a novel porphyrin with chloro*per*-fluoropolyether substituents at the *meso* position. The product was then complexed with cobalt and zinc. It was found that both the free form of the compound and its complex with zinc were highly soluble in carbon dioxide at 20 MPa and 40°C. The authors then concluded that homogeneous catalysis would be able to be performed in this supercritical fluid environment.

2.4 Fluorinated Polymer Synthesis

The synthesis of fluorine-containing polymers has attracted the interest of many researchers for quite some time now. Several patents date back to the late 1960's and early 1970's describing the synthesis of fluorinated polyesters.(101-103) However, the methods employed for these patents required long reaction times (some examples were as many as five days), high temperatures, and harsh acid chlorides, which are highly toxic and not environmentally friendly.

Keller(104) prepared fluorinated polyesters from 1,3-bis(2-hydroxyhexafluoro-2-propyl) benzene and either adipoyl chloride or glutaryl chloride at 130°C for 48 hours. It was found that by using equimolar quantities of the reactants, polyesters of no appreciable molecular weights were formed. However, higher molecular weights were achieved when an excess of one reactant or the other was used. For example, when an excess of 1,3-bis(2-hydroxyhexafluoro-2-propyl) benzene was employed in the reaction, the resulting polymer had a number average molecular weight of 2111 Daltons. When an excess of the acid chloride was used, the product had a number average molecular weight of 2718 Daltons.

DeSimone et al.(105) conducted the homogeneous free radical polymerization of fluorinated acrylate monomers in supercritical carbon dioxide using azobisisobutyronitrile (AIBN) as an initiator. The studies were conducted on homopolymers of 1,1-dihydroperfluorooctyl acrylate (FOA) and copolymers of FOA with ethylene, styrene, butyl acrylate, and methyl methacrylate. The authors found that not only was carbon dioxide a suitable solvent alternative to chlorofluorocarbons for producing relatively high molecular weight polymers, but additionally, AIBN decomposes 2.5 times slower in carbon dioxide but with greater efficiency than in benzene.

DeSimone et al.(106) also performed dispersion polymerizations of methylmethacrylate in supercritical carbon dioxide by using poly (1,1-dihydroperfluorooctylacrylate) as a stabilizer. The authors also synthesized a fluorinated initiator, F-AIBN, by treating 4,4′-azobis-4-cyanopentanoyl chloride with 1,1,2,2-tetrahydroperfluorodecanol. No change was observed when F-AIBN was used in place of AIBN. However, when stabilizer was added, high molecular weight polymers were obtained as well as high conversions.

Toselli et al.(46) prepared fluorinated poly(butylene terephthalate) by copolymerizing dimethyl terephthalate, 1,4-butanediol, and a perfluoropolyether having a number average molecular weight of approximately 2200 Daltons using $Ti(OBu)_4$ as the catalyst. The first stage of the polymerization was carried out at atmospheric pressure and the temperature was raised from 160°C to 200°C. During the second stage of the polymerization, the temperature was raised further to 250°C and the pressure was reduced to 0.3mbar. The resulting polymer samples were analyzed by NMR and FT-IR. It was found that introducing the perfluoropolyether into the polymer structure resulted in a material with decreased tensile properties, increased wear resistance, and a decreased coefficient of friction.

Reddy et al.(107) synthesized a series of fluorinated acrylate polymers and copolymers from 1,3-bis(hexafluoro-2-hydroxy-2-propyl) benzene and methyl acrylate or methylmethacrylate. The polymerizations were carried out in both solution and bulk, using α,α'-azobisisobutyronitrile (AIBN) as a radical initiator. The fluorinated acrylate or methacrylate monomers were first prepared by reacting the 1,3-bis(hexafluoro-2-hydroxy-2-propyl) benzene with dimethylsulfate to give 1-(hexafluoro-2-hydroxy-2-propyl)-3-(hexafluoro-2-methoxy-2-propyl) benzene, which was subsequently reacted with acryloyl chloride or methacryloyl chloride in order to give the correct monomers. The polymers were found to form brittle films and be thermally stable up to 391°C. The glass transition temperatures of the polymers ranged from 50°C to 104°C.

Reddy et al.(108) also synthesized polyesters containing a hexafluoro-2-propoxy group by first preparing the 4-(2-hydroxyhexafluoro-2-propyl) benzoyl chloride by dichromate oxidation of 4-(2-hydroxyhexafluoro-2-propyl) toluene and reacting it with thionyl chloride. The 4-(2-hydroxyhexafluoro-2-propyl) benzoyl chloride was then dissolved in THF and then reacted with a solution of DMAP and triethylamine for 48 hours. Copolyesters were also prepared by reacting 1,3- or 1,4-bis(2-hydroxyhexafluoro-2-propyl) benzene with a diacid chloride in THF in the presence of DMAP and triethylamine. All of the polymers were highly crystalline and displayed very low solubilities in organic solvents.

Person et al.(109) synthesized 2,5-difluoroterephthalic acid via a four step process starting from difluorotoluene. The diacid was then converted into an acid chloride using thionyl chloride. Polyesters were then synthesized in THF using various diols and bisphenols. It was found that the polymers derived from aliphatic diols had higher viscosities than those made from the bisphenols. The aliphatic polyesters formed flexible films, while the aromatic ones formed brittle films. The aromatic polyesters were found to be more thermally stable (up to 480°C). However, in both cases, the inclusion of the fluorine groups appeared to lower the melting temperatures of the polymers when compared to their unfluorinated counterparts.

Ryan et al.(110) synthesized fluorinated polymers in supercritical carbon dioxide by polymerizing 1H,1H,5H-octafluoropentyl acrylate at 65°C, using AIBN as the initiator. When the monomer concentration was 0.035 g/cm^3 and the initiator concentration was 2.00 mg/ cm^3, the weight average molecular weight of the polymer was 5,000 Daltons. However, when the monomer concentration was increased to 0.083 g/cm^3 and the initiator concentration was decreased to 1.43 mg/ cm^3, the weight average molecular weight increased to 13,500 Daltons. The authors also performed the same reaction in 1,1,2-trichlorotrifluoroethane (Freon 113) for a 24 hour period and compared it to the reaction in which supercritical carbon dioxide was used as the solvent. It was found that the polymers synthesized in Freon 113 had a significantly higher molecular weight than those synthesized in carbon dioxide.

DeYoung et al.(111) synthesized copolymers consisting of tetrafluoroethylene (TFE) with either hexafluoropropylene (HFP) or perfluoropropylvinyl ether (PPVE) at 35°C and between 2000 and 2500 psi. Bis(perfluoro-2-propoxypropionyl) peroxide was used as an initiator. In the copolymers synthesized from TFE and HFP, up to 13.8 wt% of the HFP was incorporated into the polymer. The melting points of these polymers ranged between 250°C and 267°C. The copolymers synthesized from TFE and PPVE incorporated up to 8.9 wt% PPVE and had melting temperatures between 297.1°C and 321.5°C.

Levi et al.(112) synthesized polyesters containing both segments of aromaticity and segments of comprised of perfluoropolyethers. A fluorinated prepolymer was first synthesized by reacting the perfluoropolyether with terephthaloyl chloride in 1,1,2-trichlorotrifluoroethane (Freon 113) at 40°C in anhydrous pyridine. The prepolymer was then dissolved in an equivolumetric mixture of Freon 113 and dichloromethane. A given diphenol was then separately dissolved in the same volume of solvent as the prepolymer in a mixture of water and sodium hydroxide and a phase transfer catalyst. The organic solution was then added to the aqueous solution and stirred for 30 minutes at ambient temperature. All of the synthesized polyesters possessed a very low glass transition temperature (less than -110°C), which corresponded to the perfluoropolyether segment and a higher glass transition temperature, which corresponded to the aromatic segment. The value of the second glass transition temperature depended on the nature of the aromatic segment. The polymers also had large contact angles and swelled when placed in organic solvents.

Kajiyama et al.(113) synthesized aromatic polyamides and polyarylates that had perfluorinated side chains by using 5-perfluoroalkylisophthaloyl dichlorides. The polymers were synthesized via low temperature solution polycondensation and interfacial polycondensation. Polymer yields ranged from 65% to 100%. While the thermal stabilities of the polymers were relatively unchanged by the fluorine incorporation, the glass transition temperatures decreased dramatically. As more perfluoroalkyl groups were introduced, the contact angles of the polymers increased significantly for the polyarylates but only slightly for the aromatic polyamides.

Maccone et al.(114) examined the molecular weight distribution of various fluorinated polymers with long chain branching. The authors found that coupling size exclusion chromatography data with intrinsic viscosity data of the whole polymer was a reliable analysis tool for examining the molecular weight distribution of the samples. The results were found to compare favorably with those obtained from a laser light scattering technique. The authors were also able to demonstrate the effect of branching on the molecular weight distribution by fractionating the samples and analyzing the high molecular weight samples separately.

2.5 Fluorine-Containing Polymers with Pendant Hydroxyl Groups

While much literature has been published dealing with copolymers comprised of fluorinated segments,(115-119) there has been very little published on research dealing with fluorinated polymers having pendant hydroxyl groups. Polymers such as these are likely to have interesting properties and could possibly be used as compatibilizers for polymer blends. There has been no published work involving the synthesis of these materials using enzymes.

Scheirs et al.(120) synthesized perfluorinated polyethers having a variety of pendant groups, including hydroxyl groups. The functionalized polymer is prepared by reacting Fomblin® ZDOL, a dimethyl-terminated perfluorinated polyether, with glycidol at 70°C in the presence of a potassium tertbutoxide catalyst. The same perfluoropolyether was also synthesized having pendant ester and urethane groups. This was for comparative purposes in order to evaluate the photostability of the perfluoropolyether with the pendant hydroxyl groups when it is used as a prepolymer for polyester or polyurethane synthesis. All polymer samples were exposed to UV light of wavelengths greater than 300ηm in air. It was found that the polyether sample with the pendant hydroxyl groups was found to undergo rapid photooxidation, with the final oxidation product being that of a polymer of fluorinated ether segments linked together by an ester bond. However, when the polyether's pendant hydroxyl groups were modified to form urethane or ester groups, the photostability of the polymers was greatly enhanced.

Thames and Gupta(121) synthesized esters that had a fluorinated segment as well as a pendant hydroxyl group. This was accomplished by first carrying out the epoxidation of secondary high molecular weight guayule rubber (SHMWGR) by adding *m*-chloroperbenzoic acid to a solution of SHMWGR in 1,2-dichloroethane at 0-5°C. Once the epoxidation was complete, the compound then underwent ring opening with various fluoroacids. The resulting products had ester linkages that were fluorinated, as well as pendant hydroxyl groups. The compounds were characterized by FT-IR, NMR, and DSC. It was shown that the glass transition temperatures increased as the content of fluoroacid was increased. However, the glass transition temperatures were decreased when longer chain fluoroacids were used. The fluoroesters were also found to be thermally stable up to temperatures as great as 400°C.

2.6 Ionic Liquids

During the past few years, a new area of research has experienced a large thrust. This area is the study of ionic liquids as potential solvent replacements for "green" technology. Several reviews have been published in journals and magazines(122-126) and new discoveries are being made everyday in this exciting field.

Huddleston et al.(127) synthesized a room temperature ionic liquid, butylmethylimidazolium hexafluorophosphate and studied the partitioning of substituted benzene derivatives between this compound and water. The partitioning in this system was similar to partitioning demonstrated in a water-octanol system, indicating that ionic liquids may be suitable replacements for organic solvents in liquid-liquid extraction processes.

Chauvin et al.(19) studied the dimerization of propene catalyzed by nickel-phosphine complexes in another ionic liquid, 1-butyl-3-methylimidazolium chloride. The reaction took place in a two-phase solvent system, using the ionic liquid as the solvent for the catalyst. It was found that when the ionic liquid was more basic in nature, the catalyst retained no activity. However, when the ionic liquid was acidic in nature, the catalyst was stabilized. The alkenes were easily separated from the ionic liquid phase, which retained the catalyst.

Dullius et al.(20) performed the hydrodimerization of 1,3-butadiene catalyzed by palladium (II) compounds immobilized in the ionic liquid, 1-*n*-butyl-3-methylimidazolium tetrafluoroborate. It was found that the activity of the catalyst and selectivity of the products formed were dependent on the reaction conditions. However, usually 1,3-butadiene dimer, 1,3,6-octatriene, and octa-2,7-dien-1-ol were the only products formed. The conversion of 1,3-butadiene was enhanced when carbon dioxide, pressurized to 5 atm, was added to the reaction system. All reactions were carried out at 70°C but it was discovered that the products could be decanted off at temperatures less than 5°C due to the fact that at these temperatures, the reaction system split into two phases. The solution of the ionic liquid and the catalyst was also found to be reusable.

Crisp et al.(21) investigated the reduction of dioxotetrachlororuthenate (VI) to hexachlororuthenate (IV) in the ionic liquid, 1-butylpyridinium chloride aluminum (III) chloride. The anion, $[RuO_2Cl_4]^{2-}$, was found to be stable in the ionic liquid long enough at ambient temperature for its electronic absorption spectrum to be recorded, which was a first for this particular anion.

Abdul-Sada et al.(128) studied the removal of oxide impurities from chloroaluminate (III) ionic liquids with phosgene at ambient temperatures. The reaction was monitored using NMR and mass spectrometry. This was found to be an effective means of treatment for both acidic and basic forms of the ionic liquid. The oxide impurities were removed by means of the formation of carbon dioxide.

Bowlas et al.(129) studied the physical properties of a series of ionic liquids comprised of either imidazolium or pyridinium salts. The alkyl group was varied between C_2H_5 and $C_{18}H_{37}$. The authors found that when the salts of the imidazolium or pyridinium having alkyl groups of twelve carbons or more were associated with the counter ions tetrachlorocobaltate (II), tetrachloronickelate (II), or chloride, the salts were thermally stable and demonstrated liquid crystalline behavior. All chloride salts were synthesized at 100°C for 7 days. All salts were characterized through NMR, L-SIMS, IR, and elemental analysis. The melting points of the salts were also determined using DSC and polarizing optical microscopy.

Blanchard et al.(130) studied the extraction of naphthalene from the ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate using supercritical carbon dioxide. The authors found that carbon dioxide dissolved readily into the ionic liquid. However, the ionic liquid did not dissolve into the carbon dioxide. Therefore, the naphthalene was able to be extracted from the ionic liquid into the carbon dioxide without the CO_2-rich phase becoming contaminated with ionic liquid. It was also discovered that the dissolution of carbon dioxide into the ionic liquid was a reversible process due to the fact that once the naphthalene was extracted and the system depressurized, pure ionic liquid remained.

Recently, ionic liquids have been used in the field of biocatalysis as reaction media.(23,24) Erbeldinger et al.(131) performed the thermolysin-catalyzed synthesis of *Z*-aspartame in an ionic liquid. The authors observed a reaction rate that was competitive with enzymatic synthesis in an organic solvent, as well as improved enzyme stability. Similarly, Lau et al.(132) found that *Candida antarctica* could catalyze alcholysis, ammoniolysis, and perhydrolysis using ionic liquids as the solvent and that the rates were comparable, or in some instances, better than, those in organic media.

Laszlo et al.(133) studied the α-chymotrypsin catalyzed transesterification of *N*-acetyl-L-phenylalanine ethyl ester with 1-propanol in two different ionic liquids and in combination with supercritical carbon dioxide. The enzyme was freeze-dried with KCl, K_2HPO_4, or poly(ethylene glycol). Interestingly, the enzyme displayed no activity in the ionic liquids, unless at least 0.25% water was added to the reaction. However, when the ionic liquids were combined with the carbon dioxide, the enzymes were active without the addition of water.

2.7 Use of Carbon Dioxide as a Viscosity Modifier

Although supercritical carbon dioxide has gained much attention in the past decade as a potential replacement for organic solvents due to its low toxicity and wide abundance, there is also much research being conducted in the area of using carbon dioxide as a viscosity modifier in polymer solutions. The viscosity reduction in polymer melts is of great importance in the manufacturing of composites and extrusion and foaming processes.

Xiong and Kiran(134) studied the viscosity of poly(dimethylsiloxane) in supercritical carbon dioxide in the temperature range of 300 to 460K. Specifically, the authors studied the dependence of viscosity on temperature, pressure and density. It was found that the dependence of viscosity on temperature was Arrhenius in nature. Similarly, it was also discovered that pressure and density dependence exhibited exponential relationships with viscosity. The authors determined that the Doolittle-type free-volume based relationship best described the viscosity data they collected.

Elkovitch et al.(135) investigated the reduction of viscosity in polystyrene via injection of supercritical carbon dioxide. The polystyrene was placed in an extruder and carbon dioxide entered the cell at 3000 psi (20.7MPa). The process was carried out at 170°C. The pressure was then immediately decreased in order to ensure that just enough carbon dioxide was delivered to the polymer in order to cause a reduction in viscosity. Once the process reached steady state, approximately 590 psi (4.1MPa), the viscosity calculations were made. The results indicated that at low RPM's the viscosity of the polymer solution could be reduced by as much as 56% by adding carbon dioxide.

Elkovitch et al.(136) also studied using supercritical carbon dioxide as a viscosity modifier in order to facilitate polymer blending between a high viscosity polymer, poly(methylmethacrylate), PMMA, and a low viscosity polymer, polystyrene. The ratio of the viscosities of these two polymers is approximately twenty. The blending of the two polymers was carried out at 2000 psi (13.7MPa) in a high pressure, stirred vessel. It was found that the carbon dioxide possessed a great affinity for the PMMA, thus reducing its viscosity to one that was comparable to that of polystyrene. The blending of these two polymers could then be more easily carried out.

Lee et al.(137) investigated the effects of supercritical carbon dioxide on the viscosity and morphological properties for blends of polystyrene and polyethylene. The experiments took place in a twin-screw extruder with the carbon dioxide completely dissolved in the polymer blend. The speed of the screw was maintained at 50 rpm and the temperature of the solution was kept between 193°C and 196°C. The concentration of carbon dioxide in the system was always 5 wt% or less. It was found that the addition of supercritical carbon dioxide into the system led to a dramatic reduction in the viscosity of the solution. The authors also varied the pressure and content of the carbon dioxide to investigate the effects of these parameters on the morphology of the polymers. At high pressures and carbon dioxide concentrations, smaller cell sizes were observed and the polystyrene domains were elongated and cylindrical. Additionally, with increasing carbon dioxide content, the size of the domains in the dispersed polystyrene phase decreased. The authors then extended this work by also looking at a single-screw extruder configuration as well.(138)

Khan et al.(139) also demonstrated that dissolved carbon dioxide in polystyrene melts helps to reduce the overall viscosity of the melt, thus allowing for supercritical carbon dioxide to be used as a processing aid for polymers. The authors concluded that these phenomena occurred due to the dilution of chain entanglements and an increased free volume in the melt. The modified free volume theory and Sanchez-Lacombe equation of state were used to describe the viscosity data obtained.

Yeo and Kiran(140) studied the reduction in viscosity of polystyrene solutions in toluene using supercritical carbon dioxide. Various concentrations of carbon dioxide were added to the polymer solutions (the concentration of polystyrene in toluene was 1.5 wt%) up to 14.0 wt%. The authors found that viscosity decreased exponentially with the addition of carbon dioxide and that the results could be correlated via a simple relationship.

3.0 RESEARCH OBJECTIVES

Concern for the environment is an issue that has occupied the minds of scientists for quite some time. Therefore, an area of intense research has been how to replace conventional chemical processes, solvents, and catalysts with ones that are more environmentally friendly. This has been approached in a variety of ways, two of which are biocatalysis and replacing organic solvents with either supercritical carbon dioxide, ionic liquids, or no solvent at all.

This thesis project originally began with the intent of rationally designing a continuous method for the enzymatic synthesis of polyester polyol intermediates in supercritical carbon dioxide. Previous research demonstrated that polyester molecular weight and polydispersity can be rationally and predictably controlled using supercritical fluids as the reaction medium.(25) However, because many substrates exhibit poor solubility in supercritical CO_2, finding monomers with enhanced solubility in this medium became the first objective. Studies have shown that fluorinated monomers possess this trait. Therefore, fluorinated diols were used in place of their hydrogenated counterparts for the polymerizations. Phase behavior studies were conducted on each of these compounds, as well as divinyl adipate and a fluorinated polyester product in supercritical carbon dioxide to test if this hypothesis was correct. Additionally, the biocatalytic synthesis of fluorinated polyesters was studied in great detail in order to determine the factors that limited chain extension.

Because of our previous studies in the area of linear polyesters with pendant hydroxyl groups,(55) this line of research was extended into investigating the possibility of synthesizing polyesters that possessed both fluorinated segments and pendant hydroxyl groups. Materials such as these could be used as compatibilizers or surfactants. Additionally, reactions that generate linear polymers with pendant hydroxyl groups can also be investigated for enantioselectivity.

In order to take advantage of the environmental benefits of biocatalytic polymerizations, it is essential to develop a comprehensive understanding of the process. This includes defining how and why molecular weight is limited and determining what can be done to overcome this obstacle. A recent investigation demonstrated that diffusion plays a significant role in biocatalytic polytransesterification and that the mass transfer limitations that occur in the 3,000 to 5,000 Da molecular weight range are a result of the solution viscosity being too high.(141) Therefore, another aspect of this project was to utilize supercritical carbon dioxide in a different capacity: as a viscosity modifier. Carbon dioxide was present during the reaction in order to assess what effect, if any, it had on the achievable molecular weight of the polymer. The effect of CO_2 on the enzyme was also examined.

The final objective of this project was to evaluate the applicability of ionic liquids in biocatalysis. Because they possess no measurable vapor pressure, ionic liquids represent another attractive alternative to organic solvents. Therefore, it was necessary to study enzyme activity and stability in this new medium, as well as monomer solubility in order to determine the feasibility of carrying out enzymatic polymerizations in ionic liquids. The stabilities of two lipases were examined and compared to their stability in a traditional organic solvent. These experiments were performed with and without the presence of substrates. Finally, after determining that both monomers were soluble and that the enzymes were extremely stable in this environment, biocatalytic polymerizations between divinyl adipate and 1,4-butanediol were accomplished in the ionic liquid.

4.0 EXPERIMENTAL METHODOLOGY

4.1 Enzymes

Novozym® 435 was a gift from Novo Nordisk Bioindustries (Denmark). This is a lipase from *Candida antarctica*, immobilized onto a macroporous acrylic resin. Porcine pancreatic lipase was purchased from Sigma (St. Louis, MO). Two lipase screening kits were used. The first was purchased from Amano Pharmaceutical Co. (Japan) and contained the following lipases: *Candida rugosa, Rhizopus oryzae, Penicillium camemberti, Mucor javanicus, Pseudomonas fluorescens, Aspergillus niger, and Pseudomonas cepacia*. The second kit was purchased from Biocatalytics (Pasadena, CA) and contained the following lipases: *Candida anarctica* fraction B (L2), *Candida rugosa* (L3), *Candida antarctica* fraction A (L5), *Pseudomonas sp.* (L6), porcine pancreatic lipase (L7), *Thermomyces sp.* (L8), *Mucor miehei* (L9), and *Alcaligines sp.* (L10). The kit also contained two esterases (E1 and E2) from pig liver. The high-temperature enzymes screened were from the Esterase/Lipase Clonezyme library and were purchased from Diversa, Inc. (San Diego, CA).

4.2 Materials

Divinyl adipate (DVA) was a kind gift from Union Carbide Corporation (Danbury, CT) and was also purchased from Polysciences, Inc. (Warrington, PA). All fluorinated diols were purchased from Lancaster Synthesis (Windham, NH), with the exception of 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol (OFOD), which was purchased from Oakwood Products (West Columbia, SC). All other diols and triols were purchased from Aldrich (St. Louis, MO). Sodium hydroxide beads were purchased from Fisher Scientific (Pittsburgh, PA). The ionic liquid was synthesized by the procedure described by Huddleston et al.(127) The gases carbon dioxide and nitrogen were purchased from Praxair (Oak Brook, IL) and both had purities of greater than 99.9%. All other chemicals, reagents and solvents were also obtained from Aldrich.

4.3 Experimental Procedures

4.3.1 Polymerizations in Wheaton Vials

In most reactions, equimolar amounts of divinyl adipate (DVA) and diol/triol were weighed out and placed in a Wheaton glass vial. For the solvent-free reactions, the vial was then placed in a New Brunswick Scientific Series 25 incubator/shaker and incubated at 50°C for 30 minutes. After the DVA was completely melted, enzyme was added to the vial to start the reaction. The vial was then placed back in the incubator at 50°C and a shaking speed of 250 rpm for the designated reaction time. Once the reactions were completed, the enzyme was filtered off and any excess solvent was evaporated to give the final polyester. The polyesters were analyzed without any further purification. All polyester products were soluble in tetrahydrofuran, thus allowing for analysis by means of gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF).

4.3.2 Phase Behavior Studies

All phase behavior studies were performed using a variable volume high pressure view cell (D.B. Robinson and Associates) (Figures 1 and 2). A known amount of divinyl adipate, diol or polymer was loaded onto the piston. An O-ring around the perimeter of the piston provided a seal that prevented mixing of the sample volume contents above the piston and the over border fluid below the piston. The quartz cylinder was smooth enough for the seal to retain its integrity as the piston moved within the quartz cylinder ("floating" piston). The piston and sample were placed inside the quartz cylinder encased in a windowed stainless steel vessel. Stainless steel mixing balls were placed in the sample volume and the cell was sealed. Liquid carbon dioxide was displaced into the sample volume at the same volumetric rate that the over border fluid was withdrawn. Therefore, precise amounts of carbon dioxide could be introduced into the cell. The pressure of the system was controlled by isolating the sample volume and moving the piston up or down via the injection and withdrawal of the over border fluid. The cell was rocked for 1 hour to equilibrate the mixture. Phase transitions were determined by increasing the pressure until a single, transparent phase was attained. The pressure was then slowly decreased until the solution became "cloudy", indicative of a solid or liquid second phase appearing.

4.3.3 Polymerization in Supercritical Carbon Dioxide in the Robinson Cell

The polymerization was conducted at 50°C for 24 hours in the variable volume high pressure view cell. Equimolar amounts of divinyl adipate (DVA) and

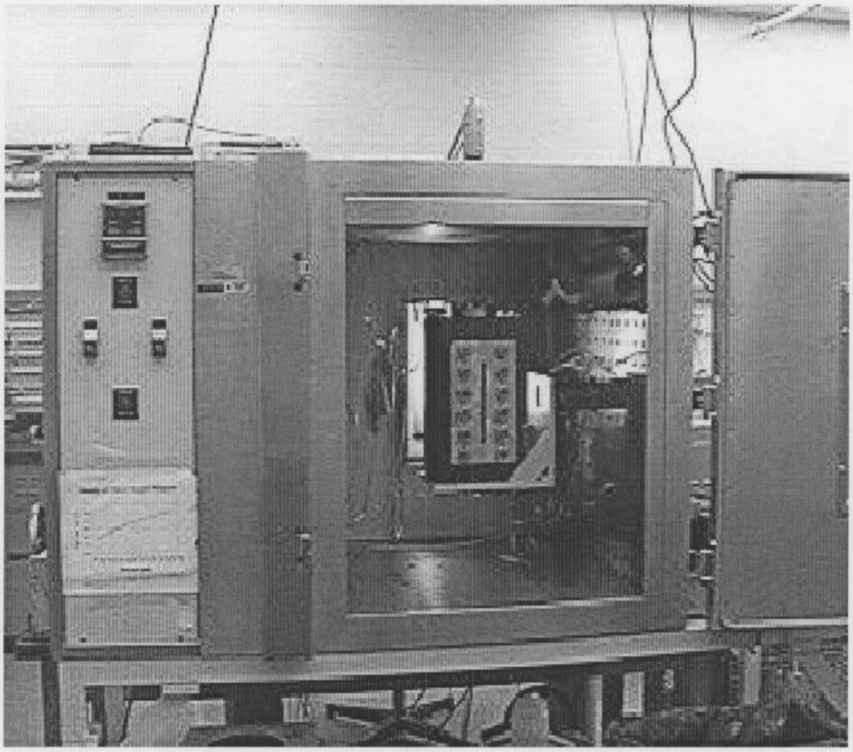


Figure 1 Digital Camera Photograph of the D.B. Robinson Cell

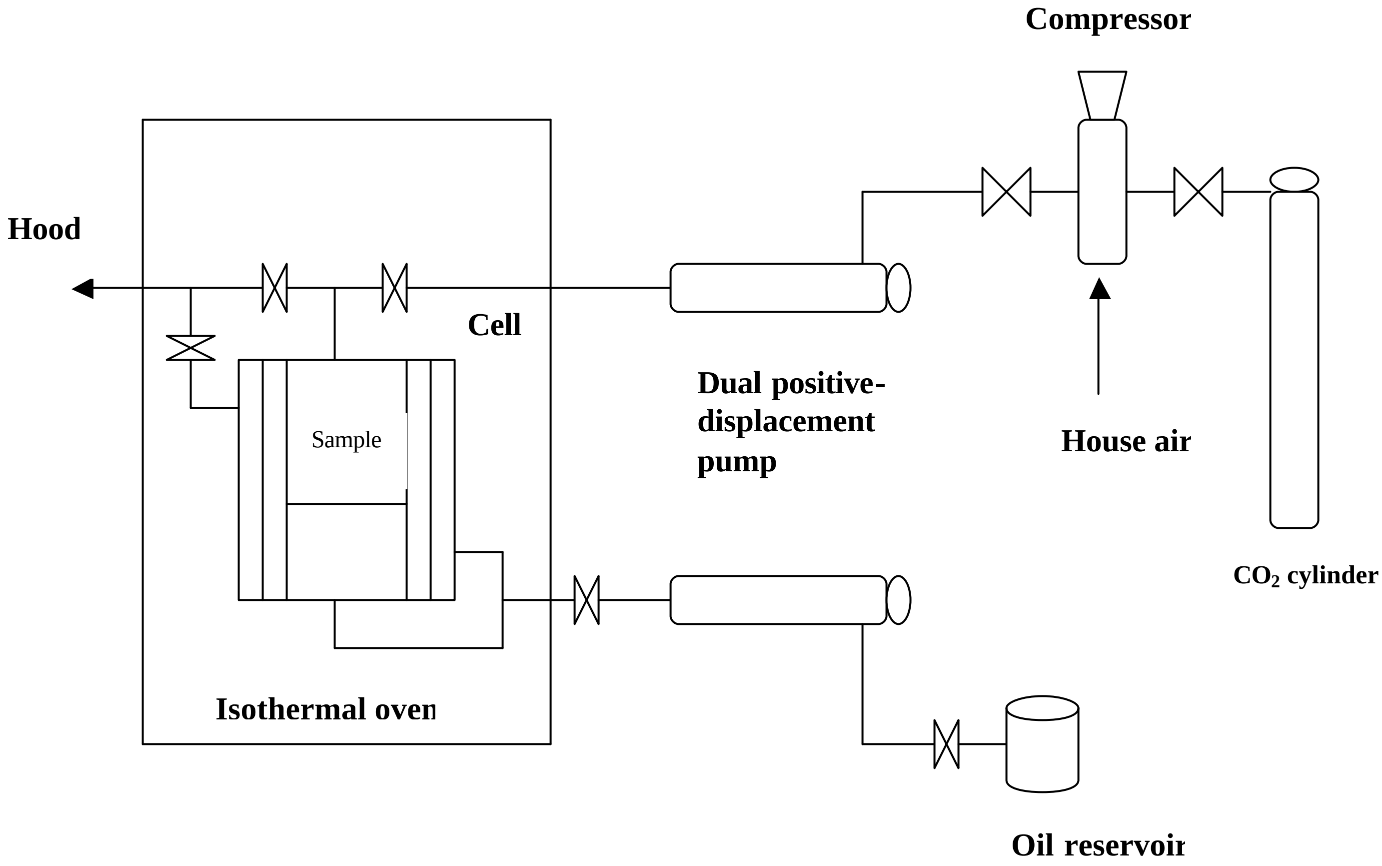


Figure 2 Schematic of the Variable Volume High Pressure View Cell

octafluorooctandiol (OFOD) were loaded onto the piston in order to achieve a final concentration of 1 wt% OFOD in supercritical carbon dioxide. Novozym® 435 was encased in a separate vial that was also loaded onto the piston, in order to prevent mixing of the substrates with the enzyme before the reaction was begun. The cell was then equilibrated at the desired temperature for 2 hours before the carbon dioxide was added. The reaction was then initiated by turning on the rocking mechanism in the cell, which allowed for the enzyme to be released from its vial. The cell was then rocked for 24 hours, after which time the carbon dioxide was vented and the product was collected and analyzed via GPC.

4.3.4 Polymerizations in the High Pressure Stainless Steel Reactor

For reactions in which the effect of carbon dioxide on polyester molecular weight was studied, a high pressure stainless steel reactor was used (Figures 3 and 4). Equimolar amounts of divinyl adipate and 1,4-butanediol were placed in the reactor and allowed to incubate for 30 minutes. Enzyme was then added to the reactor, along with a magnetic stir bar, in order to ensure proper mixing. The reactor was then sealed and carbon dioxide or nitrogen was introduced into the reactor. The reaction was allowed to proceed at the desired temperature and pressure for given amounts of time. Both temperature and pressure were monitored at all times during the reaction. At the completion of the reaction, the magnetic stir bar was turned off and the reactor was slowly vented. Once all of the gas was removed from the reactor, the lid was unscrewed and the resulting products were collected and analyzed.

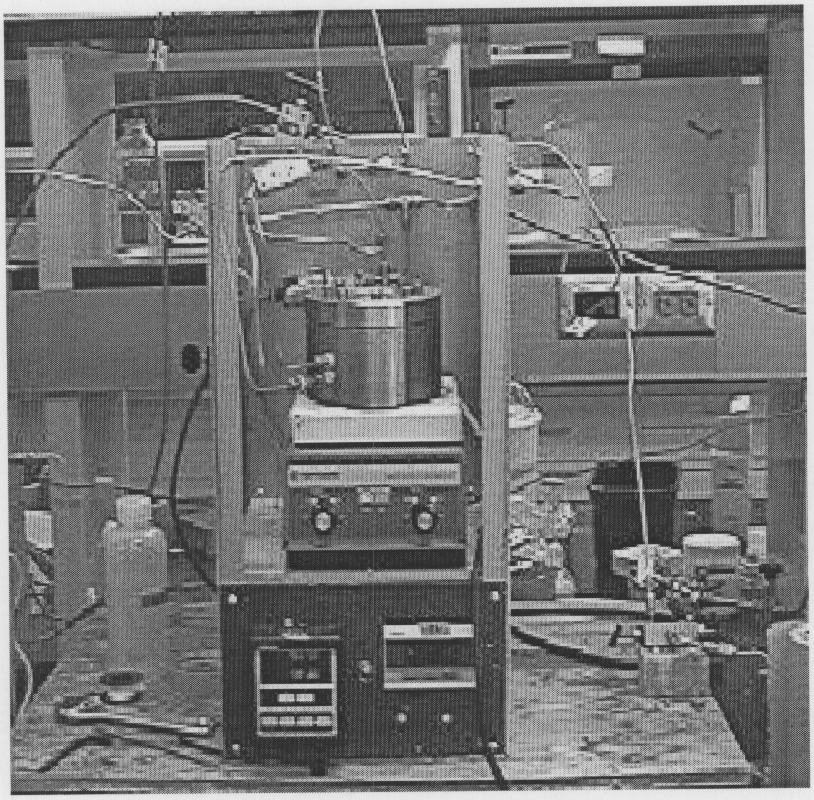


Figure 3 Digital Camera Photograph of the High Pressure Stainless Steel Reactor

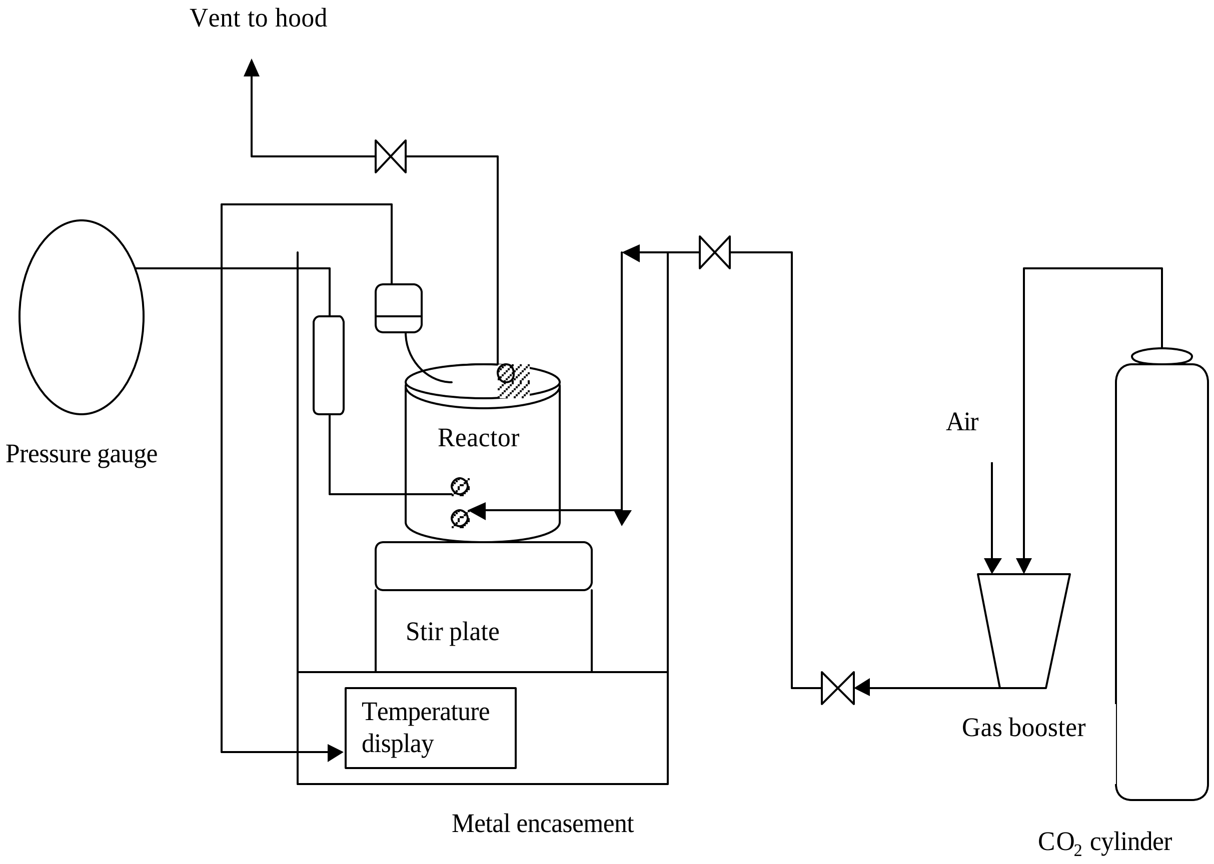


Figure 4 Schematic of the High Pressure Stainless Steel Reactor

4.4 Analysis Procedures

4.4.1 Polyester Analysis

4.4.1.1 Gel Permeation Chromatography. The molecular weights of the polyesters were determined using a Waters 150CV gel permeation chromatograph equipped with a refractive index detector. Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1.0 ml/min and 35°C. Three columns in series are installed in the instrument to achieve sufficient separation for the molecular weight range of 500-30,000 Da. The first two columns, PL-gel Mixed-E columns from Polymer Laboratories, have mixed porosity. The third column, a Waters Ultrastyragel column, has a uniform pore size of 500 Å. A calibration curve, which plotted the log of the molecular weight versus the retention time, was constructed using 11 polystyrene standards in the molecular weight range of 580-66,000 Da.

4.4.1.2 Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF). Polyester end group functionality and composition was determined using a PerSeptive Biosystems Voyager Elite MALDI-TOF mass spectrometer. The spectrometer was operated at a voltage of 20 kV in linear mode. A 10 μg/ml solution of sodium iodide in water was prepared and spotted onto the sample plate and allowed to dry before the actual samples and matrix were spotted. A solution of the polyester and matrix was then prepared using 18μl of dithranol (5mg/ml in THF) and 2μl of polyester (20mg/ml in THF). Approximately 1.5-2μl of this solution was then spotted onto the sample plate once the sodium iodide solution was completely dried. Once the sample plate was completely dry, it was then loaded into the instrument, where the samples were then analyzed. The spectra were calibrated using peptide standards obtained from PerSeptive Biosystems (Framingham, MA) in an α-cyano-4-hydroxy cinnamic acid matrix. The peptides in the calibration mixture included des-ARG-bradykinin, angiotensin I, Glu-fibrinopeptide B, and neurotensin.

4.4.1.3 Hydroxyl Content. The polyester hydroxyl number was determined using a titrimetric technique developed by Bayer Corp. (Miles' Quality Laboratories, Method Number 414-C). A sample of polyester was reacted with an excess of phthalic anhydride in pyridine for 35 minutes at approximately 182ºC. The unreacted phthalic anhydride was then titrated with a standardized 1.0 N solution of sodium hydroxide to its endpoint using phenolphthalein as an indicator. The amount of polyester used in the analysis varied according to what the expected hydroxyl number would be. Polyesters with lower hydroxyl numbers required a larger amount of sample for analysis.

4.4.2 Enzyme Analysis

4.4.2.1 Ionic Liquid Stability Studies. Enzyme thermostability at 50ºC was measured titrimetrically using a Sigma diagnostic kit (800-B). 10 mg of enzyme were incubated at 50ºC in either the ionic liquid or organic solvent both alone and in the presence of monomer for given amounts of time, after which the ionic liquid/organic solvent was removed. The enzyme was then incubated with 3 ml of substrate from the kit for 2 hours at 50ºC in a shaking incubator. Enzyme activity, which is expressed as Sigma/Tietz Units/mg, was estimated by determining the amount of fatty acid produced.

4.4.2.2 High Pressure Stability Studies. For the stability studies of the enzyme in high pressure carbon dioxide or nitrogen, the same procedure was followed as in Section 4.4.2.1. The enzyme was placed in the high pressure stainless steel reactor for given amounts of time in the presence of either carbon dioxide or nitrogen. At the end of the designated time, the reactor was slowly vented and the enzyme removed. Samples were then treated accordingly with the Sigma/Tietz Assay Kit.

4.4.2.3 Electron Microscopy. Electron microscopy of enzyme samples was performed by the Department of Cell Biology and Physiology in the Bioscience Tower at the University of Pittsburgh School of Medicine using a JEOL 6550 SEM (JEOL, Peabody, MA). Samples of Novozym® 435 that were exposed to either nitrogen or carbon dioxide for various amounts of time were analyzed via this method. The enzyme beads were attached to SEM stubs by double-sided sticky copper tape. The samples were overcoated with 3ηm of gold/palladium using a Cressington 108 Auto Sputter Coater (Cressington Scientific Instruments, LTD, Watford, UK).

4.4.2.4 BET Analysis. BET analysis of the enzyme samples was performed by Materials Testing Laboratory (Pennington, NJ). Novozym® 435 samples were analyzed to determine if there was a decrease in bead porosity over time after being exposed to high pressure nitrogen and carbon dioxide.

5.0 BIOCATALYTIC SYNTHESIS OF FLUORINATED POLYESTERS

5.1 Objective and Introduction

The biocatalytic synthesis of fluorinated polyesters was studied for several reasons. In order to increase monomer solubility in supercritical carbon dioxide, fluorinated diols were used in place of their hydrogenated counterparts. This would then allow for polyester synthesis to be conducted in supercritical carbon dioxide and permit the rational control of polyester molecular weight and dispersity by simply changing the pressure of the system. Polyester polyols are industrially relevant because they are used as intermediates for the polyurethane industry. Synthesizing these compounds via conventional chemical catalysis requires the use of high temperatures and the constant removal of water. This is compounded by the fact that the catalysts used to synthesize the polyols can have undesirable effects on the ensuing polyurethane reactions. Enzymes require mild reaction conditions to function and possess high selectivity. Additionally, by using supercritical fluids as the reaction medium, enzyme activity and selectivity can be controlled through changes in pressure or temperature. Supercritical fluids also allow for solvent tunability, as well as enhancement of mass transfer rates. Carbon dioxide is particularly attractive because it possesses low toxicity, is widely available, and is classified as a non-volatile organic chemical (VOC).

Fluorinated polymers are commercially relevant and have some unique properties. Their synthesis allows for materials with low water absorption, low coefficients of friction, low surface energies, and improved chemical resistance.(38-41) The surface properties of fluorinated polyesters make them attractive for use in the biomaterials industry(41) and fluorinated hydroxyl-terminated polyesters have also been used as polyurethane prepolymers.(42) However, previous research has shown that these materials can be relatively difficult to synthesize by traditional chemical methods.

Therefore, if fluorinated polyesters are to by synthesized enzymatically, it is of utmost importance to study the polymerization process in great detail and understand what molecular weights are achievable and what factors could possibly limit chain extension. Thus, the Novozym® 435-catalyzed reaction between divinyl adipate and fluorinated diols of varying chain length was examined (Figure 5). The thermodynamic equilibrium in Figure 5 is shifted in the forward direction by using divinyl adipate as one of the monomers.(142,143) The byproduct of the reaction is vinyl alcohol, which instantaneously tautomerizes to acetaldehyde. The fluorinated diols used in these studies are summarized in Table 2. By observing trends in polymer molecular weight growth and end group functionalities, as well as determining what could limit chain extension, a more detailed understanding of this process can be gained.

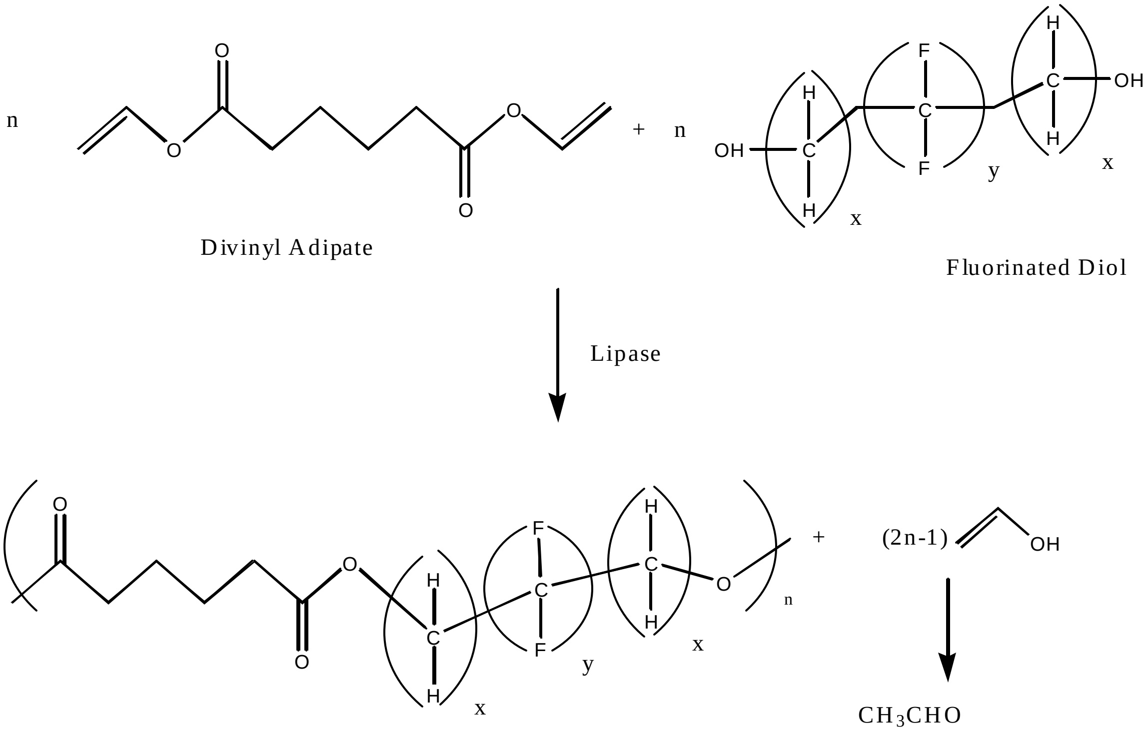
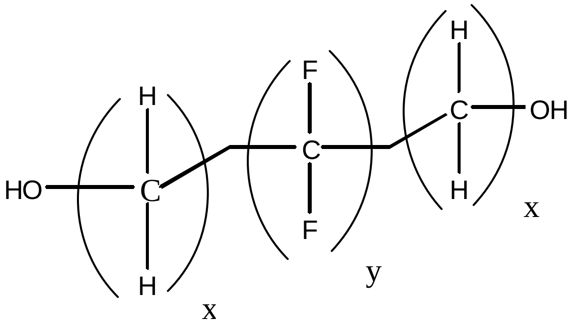


Figure 5 Schematic of Lipase-Catalyzed Fluorinated Polyester Synthesis

Table 2 Fluorinated Diols Studied



Name of Compound	Abbreviation	x	y
2,2,3,3-Tetrafluoro-1,4-butanediol	TFBD	1	2
2,2,3,3,4,4-Hexafluoro-1,5-pentanediol	HFPD	1	3
1H, 1H, 9H, 9H-Perfluoro-1,9-nonanediol	PFND	1	7
1H, 1H, 12H, 12H-Perfluoro-1,12-dodecanediol	PFDD	1	10
3,3,4,4,5,5,6,6-Octafluorooctan-1,8-diol	OFOD	2	4

* x and y refer to the subscripts in the above figure

5.2 Procedure

All reactions were performed in Wheaton vials of varying sizes, depending on the amounts of monomers used. For the bulk reactions, the monomers were incubated at 50°C for 30 minutes, after which the enzyme was added to the vial. At the completion of the reactions, the products were isolated and analyzed without further purification via gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). The reactions between DVA and TFBD and DVA and OFOD produced a white, amorphous solid polyester, whereas the reaction between DVA and HFPD produced an opaque, viscous liquid polyester. Once an enzyme screen was performed in order to conclude which enzyme was the most effective in producing polyester, the effects of time, continuous enzyme addition, enzyme concentration, and diol chain length were studied in order to determine the factors that limited chain extension, such as enzyme inactivation, enzyme specificity, the equilibrium position for the reaction, hydrolytic side reactions, and polymer precipitation.

5.3 Enzyme Screen

An enzyme screen was performed to select an enzyme that could successfully catalyze the synthesis of fluorinated polyesters from fluorinated diols and divinyl adipate. Eight mesophilic lipases were tested with each of the fluorinated diols in a Freon 113/acetone (74%/26%) solvent system (TFBD, HFPD, PFND, and PFDD were all soluble in this mixture). All of the enzymes tested failed to produce polymer for both the reaction with DVA and PFND and the reaction with DVA and PFDD. However, Novozym® 435 was found to be effective in the reactions between DVA and TFBD and DVA and HFPD. The results of the enzyme screen can be seen in Table 3.

Table 3 Enzyme Screen for the Reactions between DVA and TFBD/HFPD

Enzyme	Substrate 1	M_w	# of Repeat Units	PDI
Ps. fluorescens	TFBD	740	2	1.12
C. rugosa	TFBD	602	2	1.07
R. oryzae	TFBD	564	2	1.19
P. camemberti	TFBD	465	2	1.03
M. Javanicus	TFBD	567	2	1.04
Ps. cepacia	TFBD	949	3	1.36
Porcine pancreatic lipase	TFBD	537	2	1.05
C. antarctica	TFBD	2590	6	1.65
C. antarctica	HFPD	2146	3	2.10

Reaction conditions: substrate 2 was DVA in all cases; [DVA] = [TFBD]/[HFPD] = 0.45M; wt% of all enzymes was 2%(w/v); solvent used was a mixture of 26% acetone and 74% Freon 113; reaction time = 24 hours; reaction temperature = 50°C. Note: number of repeat units was calculated using M_n.

A high temperature enzyme screen was also performed using thermostable lipases and esterases purchased from Diversa, Inc. Although four enzymes were tested with the DVA/TFBD substrate system at 90°C, the highest weight average molecular weight produced was 831 (PDI = 1.71; number of repeat units = 2). When the same reaction was performed at 80°C, the weight average molecular weight increased slightly to 1165 (PDI = 1.63; number of repeat units = 3).

5.4 Limiting Factors in Chain Extension

As demonstrated by the enzyme screen, Novozym® 435 was clearly the best enzyme to study further. The reaction between DVA and TFBD was performed in tetrahydrofuran (THF) with 2%(w/v) Novozym® 435 at 50°C for 24 hours. However, the molecular weight of the resulting fluorinated oligomer was significantly lower than that in the Novozym® 435-catalyzed reaction between DVA and 1,4-butanediol (BD) performed under the same reaction conditions (Table 4).

Table 4 Polyester Molecular Weight Comparison

Reaction	M_w	PDI	# of Repeat Units
DVA + BD	9440	3.06	15
DVA + TFBD	1773	1.65	4

Reaction conditions: reaction time = 24 hours; reaction temperature = 50°C; solvent used was THF; enzyme concentration = 2%(w/v).

To further understand the limiting factors in chain extension, the effects of various reaction conditions on the polymerization process were evaluated. For the Novozym® 435-catalyzed polymerization of DVA and TFBD in THF, molecular weight increased linearly with time until approximately 30 hours, after which further increases in molecular weight became very small (Figure 6). Early termination of chain extension could result from a combination of enzyme inactivation, enzyme specificity, the equilibrium position for the reaction, hydrolytic side reactions, and polymer precipitation.

5.4.1 Enzyme Inactivation

Enzyme inactivation was ruled out by replacing enzyme every 24 hours with fresh catalyst once the initial 30 hour period of increasing molecular weight was complete. For this set of experiments, the old enzyme was not removed. No further increase in molecular weight was observed (Figure 7). In addition, another set of experiments was performed in which the old enzyme was removed by filtration when fresh catalyst was added. However, molecular weight still did not increase. Therefore, the limiting factors in chain extension did not include enzyme inactivation.

5.4.2 Hydrolytic Side Reactions

It was also unlikely that the limiting factor in chain extension was a hydrolytic side reaction, as has been hypothesized for polyester synthesis,(69) because MALDI-TOF analysis of the fluorinated polyester showed that the majority of end groups were hydroxyl-hydroxyl, vinyl-hydroxyl, and vinyl-vinyl. The presence of vinyl end groups indicated that hydrolysis was not occurring.

However, because it has been previously demonstrated for polyesters(68) that solvent type is an important governing factor in determining polymer molecular weight, the amount of water associated with the reaction was minimized. Polar solvents tend to

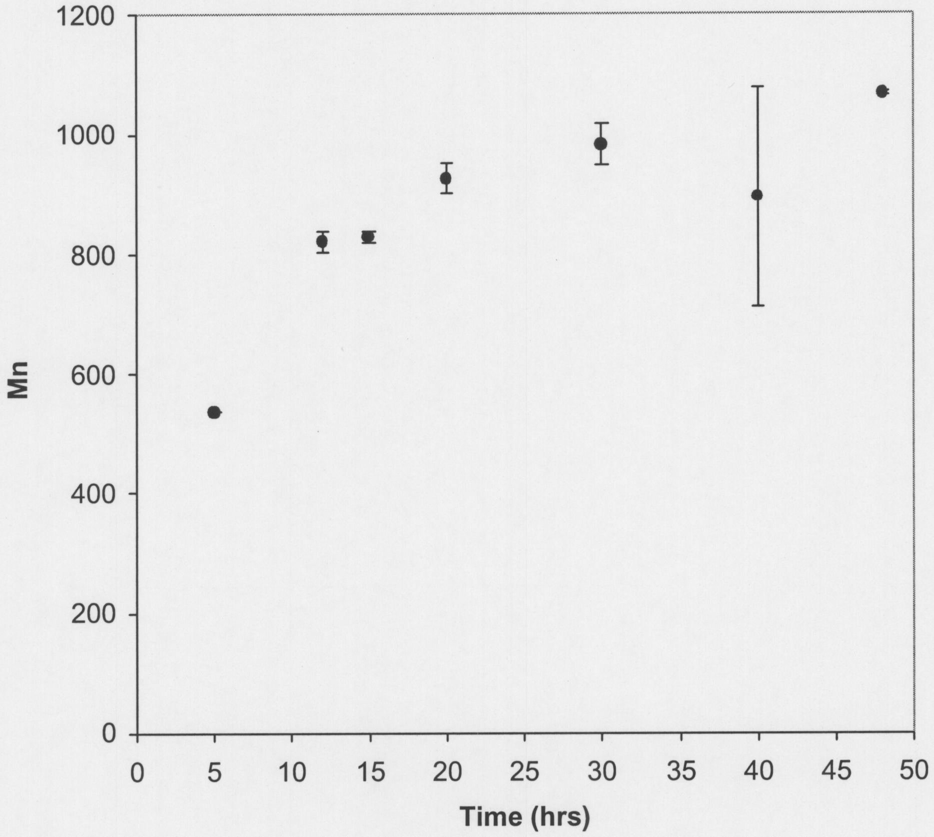


Figure 6 Time Dependence of Fluorinated Polyester Synthesis

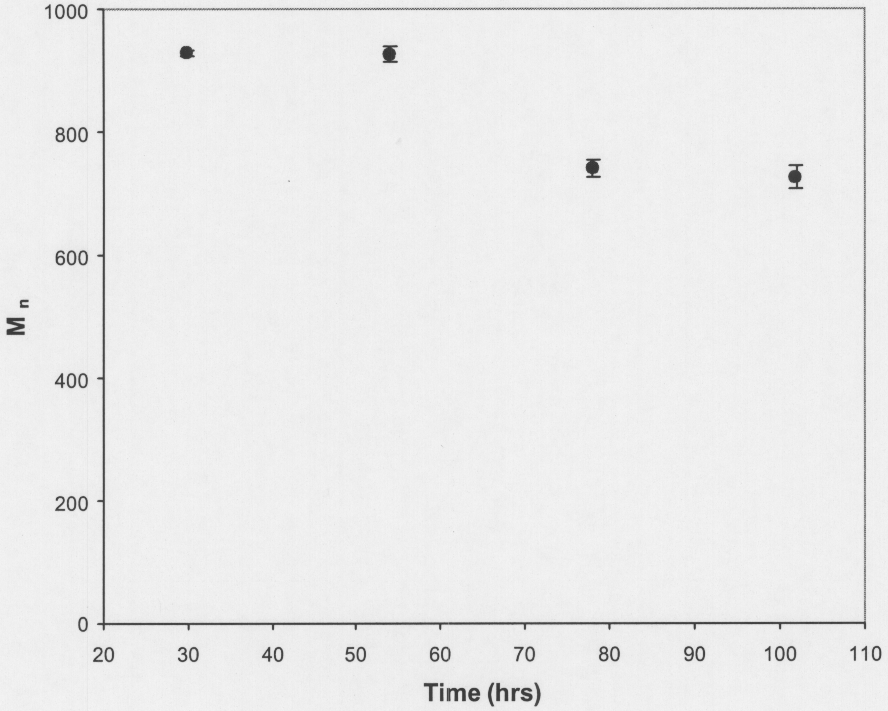


Figure 7 Effect of Continuous Enzyme Addition

partition the water bound to the enzyme into the solvent, thus leading to hydrolysis. Performing the reactions in the absence of solvent can help to minimize this problem if the substrates are hydrophobic. Therefore, a set of bulk reactions was performed with either TFBD or HFPD and DVA. The use of bulk monomer increased polymer molecular weight, although a higher enzyme concentration was required (Table 5). At lower enzyme concentrations, polymer molecular weight decreased. However, the same time-dependence trend was still observed (Figure 8).

Table 5 Solvent-Free Reactions between DVA and TFBD/HFPD

Substrates	Time (hrs)	M_w	PDI	# of Repeat Units
DVA/TFBD	24	3042 ± 269	1.58 ± 0.08	7 ± 1
DVA/TFBD	48	4603 ± 58	1.81 ± 0.11	9.5 ± 0.5
DVA/HFPD	24	6844 ± 523	2.42 ± 0.19	8.5 ± 1.5
DVA/HFPD	48	6170 ± 251	2.33 ± 0.01	8 ± 1

Reaction conditions: [DVA] = [TFBD]/[HFPD] = 7 mmol; shaker temperature = 50°C; wt% of all enzymes was 12%(w/w). Note: number of repeat units was calculated using M_n.

5.4.3 The Equilibrium Position

Although the reaction should be essentially irreversible, it is possible that some solvents can partially stabilize vinyl alcohol. Therefore, it was important to ensure that polymer molecular weight was not limited by the pseudo-equilibrium position. The addition of more enzyme would be expected to not affect the achievable molecular

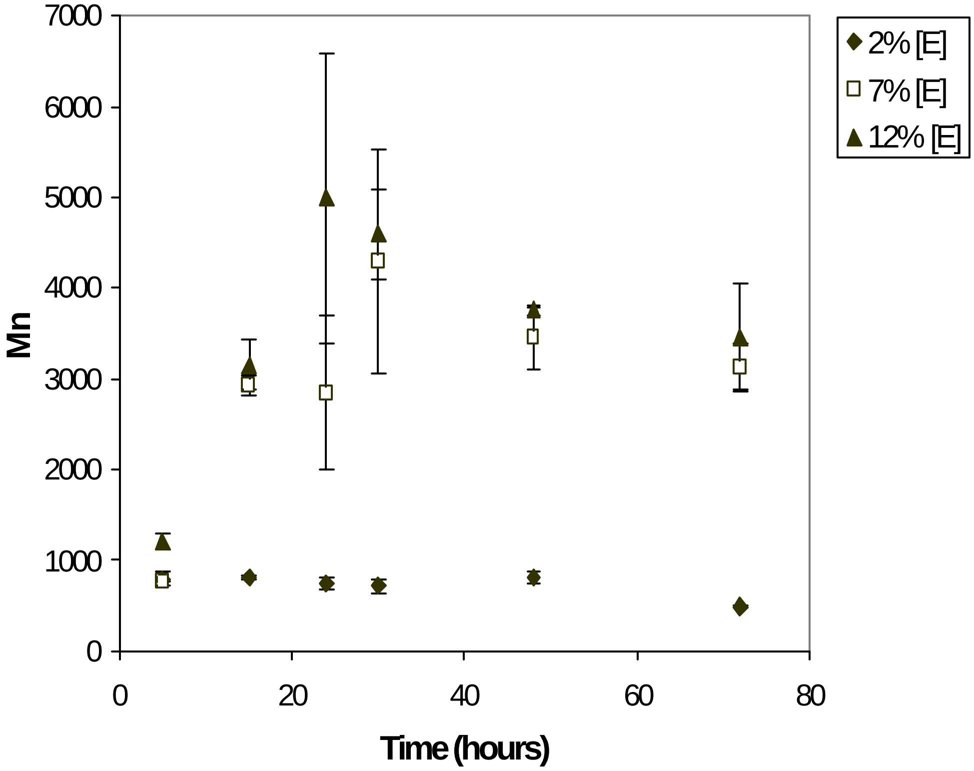


Figure 8 Time Dependence of Number Average Molecular Weight at Various Enzyme Concentrations for the Solvent-Free Reaction of DVA with HFPD

weight if the polymerization were equilibrium limited. A set of experiments was performed in which the enzyme concentration was increased from 2 to 5 wt%(w/w). Polymer molecular weight did increase slightly and the time taken to achieve maximum molecular weight did not change with the increased enzyme concentration (Figure 9). Therefore, it was concluded that the reaction was not likely limited by the equilibrium position.

5.4.4 Polymer Precipitation

Working with the Freon 113/acetone solvent system also allowed for the investigation into whether the fluorinated polyester was precipitating out of the solvent mixture. At the start of the reaction between DVA and TFBD, both monomers were soluble in the Freon 113/acetone solvent system and the reaction mixture was clear. Conversely, when the reaction was stopped, the mixture had turned white. Furthermore, when the enzyme was filtered off and the solvent was evaporated, no polymer was present in the filtrate. However, when the enzyme was washed with THF and filtered, the synthesized polymer was recovered in the THF filtrate. For the reactions in which THF was used as the solvent, the reaction mixture remained clear throughout the course of the reaction and the polymer was easily recovered. Therefore, it is more beneficial to perform the reaction in THF instead of the Freon 113/acetone mixture, provided that both monomers are soluble in THF. Additionally, all of the other fluorinated diols used remained soluble in the Freon 113/acetone solvent mixture throughout the course of the reaction. Thus, it was concluded that polymer precipitation was only a factor in the reaction between DVA and TFBD in the Freon 113/acetone solvent system.

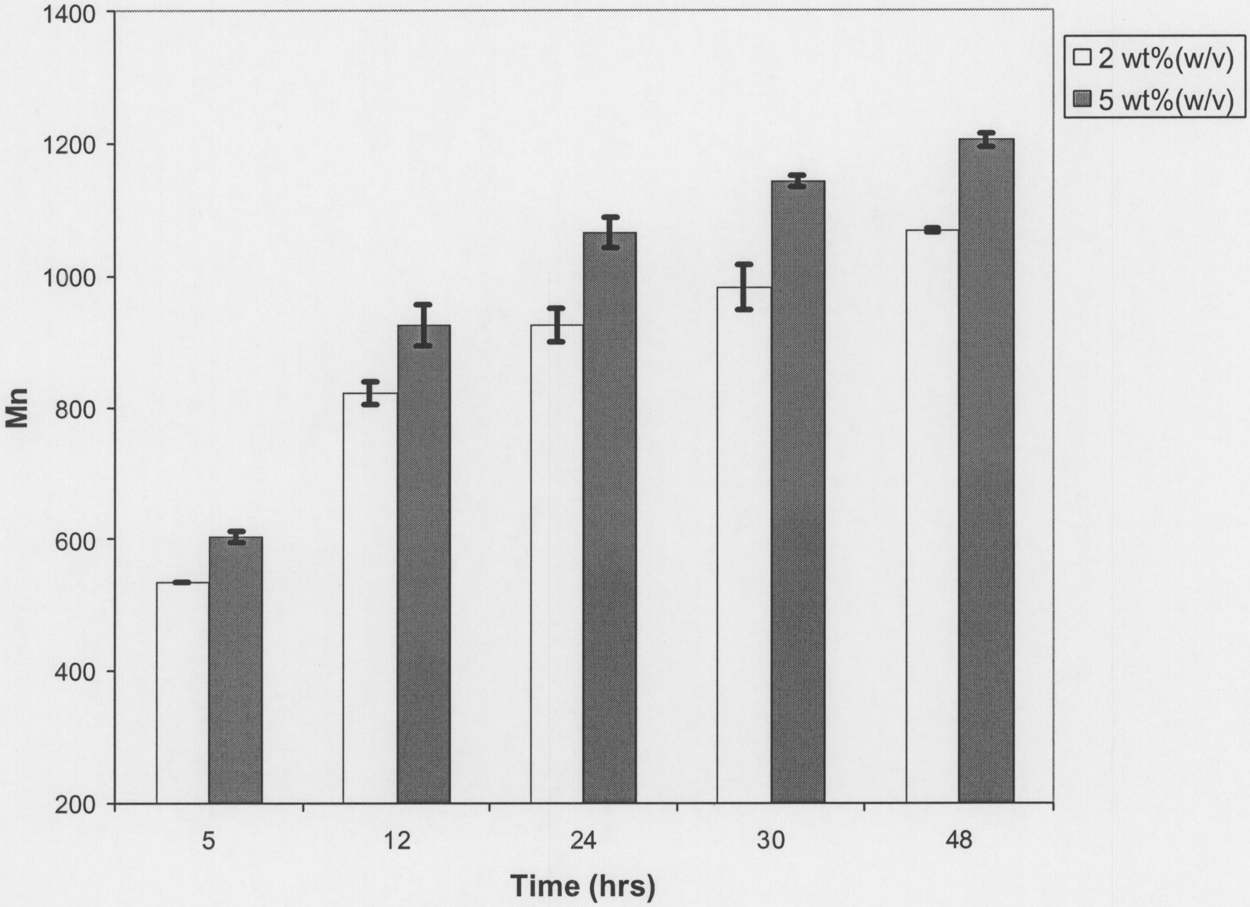


Figure 9 Effect of Increased Enzyme Concentration on Polymer Molecular Weight

5.4.5 Enzyme Specificity

Because it was apparent that the intrinsic specificity of the enzyme plays an important role in limiting chain extension of fluorinated polyesters, it was essential to determine the effect of chain length on productivity. Along with TFBD, HFPD, PFND, and PFDD were selected for the reaction with DVA, but because only TFBD was soluble in THF, the Freon 113/acetone solvent system was used. The amount of acetone required varied from 17% to 26% of the total volume of solvent used in the reaction. Therefore, all reactions were performed with 26% acetone in order to directly compare the results.

The enzyme exhibited a greater activity with the fluorinated polyesters synthesized from the shorter chain fluorinated diols. No polymer was produced for the reactions in which PFND and PFDD were used as monomers. Fluorinated oligomers with weight average molecular weights of 727 were produced with HFPD. However, the fluorinated polyester with the highest molecular weight (M_n = 1095) was synthesized from TFBD.

Given the bulky nature of the fluorinated carbons, it was also investigated as to whether the enzyme exhibited any specificity toward a fluorinated diol that contained an additional methylene spacer between the fluorine atoms and the hydroxyl groups. Reactions between DVA and OFOD were performed in solvent-free and solvent-based (THF) systems. Table 6 shows that the additional methylene spacer has a significant effect on the polymerization. Therefore, it can be concluded that the enzyme exhibits specificity toward a fluorinated diol that displays an optimum combination of chain length and fluorination.

Table 6 Reactions between DVA and OFOD

Substrates	[Enzyme]	Solvent/Bulk	Mw	PDI	# of Repeat Units
DVA/OFOD	2%(w/v)	THF	3245 ± 41	1.52 ± 0.01	5
DVA/OFOD	12%(w/w)	bulk	8094 ± 936	1.70 ± 0.05	13 ± 1

Reaction conditions: [DVA] = [OFOD] = 7mmol for solvent-free reactions; [DVA] = [OFOD] = 0.45M for THF reactions; shaker temperature = 50°C; reaction time = 24 hours. Note: average number of repeat units was found using M_n.

5.5 Conclusion

The biocatalytic synthesis of fluorinated polyesters was investigated. After initially observing the trend in polymer molecular weight growth, it was determined that chain extension was being terminated. Experiments were performed to test a variety of factors, such as time, enzyme concentration, and diol chain length, which could have limited chain extension. After ruling out possibilities such as enzyme inactivation, the equilibrium position for the reaction, hydrolytic side reactions, and polymer precipitation, it was determined that the most probable governing factor in limiting polymer molecular weight growth was the specificity of the enzyme toward fluorinated diols in which chain length and fluorination are at an optimum. A maximum molecular weight (M_n) of 5189 (13 repeat units) was observed for the solvent-free reaction between DVA and OFOD.

6.0 PHASE BEHAVIOR STUDIES IN SUPERCRITICAL CARBON DIOXIDE

6.1 Objective and Introduction

The use of supercritical fluids as nonaqueous solvents for enzyme-catalyzed reactions has attracted significant attention recently because the physical properties of a reaction environment can be easily changed in supercritical systems.(7-11) For example, significant changes in the density of a supercritical fluid can be accomplished through small changes in pressure. Therefore, all density-dependent solvent properties, such as dielectric constant and solubility parameter, are also pressure-adjustable.(12,13) It has also been demonstrated that enzyme activity(14) and selectivity(15) can be predictably controlled in supercritical fluids by varying the system pressure. Therefore, performing biocatalytic reactions in supercritical media provides an attractive alternative to organic solvents.

Supercritical carbon dioxide is particularly appealing because it is a nontoxic, unregulated solvent with high availability. However, the low polarity of carbon dioxide makes it a poor solvent for most polar substrates.(144) Because it has been shown that for polyester synthesis polymer molecular weight and polydispersity can be controlled in supercritical systems by simply changing the pressure,(25) increasing substrate solubility and polyester product in supercritical carbon dioxide is of great interest. Hydrocarbon-based diols exhibit extremely low solubility in carbon dioxide. However, previous studies have indicated that perfluorinated or semi-fluorinated analogs of CO_2-insoluble polar hydrocarbons can exhibit substantial solubility with carbon dioxide.(32,33,35-37)

Because it was demonstrated that polymer can be produced enzymatically from DVA and fluorinated diols, it was essential to determine the solubility of these monomers in supercritical carbon dioxide in order to assess the feasibility of performing the polymerization in CO_2. Therefore, the CO_2-solubility of fluorinated diols of varying chain length and degree of fluorination has been studied at 323K, along with DVA. These are the same compounds that were used in the fluorinated polyester synthesis study (Table 7). Additionally, the CO_2-solubility of an equimolar mixture of DVA and the most CO_2-soluble diol was performed, as well as the solubility of a fluorinated polyester made from the reactants. A polymerization was then conducted in supercritical carbon dioxide between equimolar amounts of DVA and OFOD.

Table 7 Compounds Tested for Solubility in Supercritical Carbon Dioxide

Name	Formula	Molecular Weight
2,2,3,3-Tetrafluoro-1,4-butanediol	$HOCH_2(CF_2)_2CH_2OH$	162.09
2,2,3,3,4,4-Hexafluoro-1,5-pentanediol	$HOCH_2(CF_2)_3CH_2OH$	212.09
1H,1H,9H,9H-Perfluoro-1,9-nonanediol	$HOCH_2(CF_2)_7CH_2OH$	412.12
1H,1H,12H,12H-Perfluoro-1,12-dodecanediol	$HOCH_2(CF_2)_{10}CH_2OH$	562.15
3,3,4,4,5,5,6,6-Octafluorooctan-1,8-diol	$HOCH_2CH_2(CF_2)_4CH_2CH_2OH$	290.15
Divinyl Adipate	$CH_2CHOOC(CH_2)_4COOCHCH_2$	198.22

6.2 Procedure

The phase behavior studies were performed in the manner described in the Experimental Methodology Section (Chapter 4). The fluorinated polyester used in the phase behavior studies was synthesized by using equimolar amounts of divinyl adipate and 2,2,3,3-tetrafluoro-1,4-butanediol in tetrahydrofuran with 2 wt%(w/v) of Novozym®435, an immobilized lipase from *Candida antarctica*. The reaction was run for 24 hours at 50°C in a New Brunswick Scientific Series 25 incubator/shaker with a shaking speed of 250 rpm. Once the reaction was complete, the enzyme was filtered off and all excess solvent was evaporated. The polyester product was analyzed without further purification. Polymer molecular weight and polydispersity were determined by gel permeation chromatography using a Waters 150CV gel permeation chromatograph with a refractive index detector. Tetrahydrofuran was used as the mobile phase at a flow rate of 1.0 mL/min and 35°C. A calibration curve was constructed using 11 polystyrene standards. Once the phase behavior studies were completed on DVA, the fluorinated diols, the fluorinated polyester, and an equimolar mixture of DVA and OFOD, a polymerization between DVA and OFOD was performed in the Robinson Cell. This procedure was also described in the Experimental Methodology Section.

6.3 Binary Data

Dew point data were collected for binary mixtures containing dilute concentrations of divinyl adipate (DVA), fluorinated diols or a semi-fluorinated diol in supercritical carbon dioxide. These binary systems exhibited dew points (Figure 10) characteristic of a Type 4 system.$^{(26)}$ Figure 11 illustrates the portion of a Type 4 diagram that corresponds to the high pressure, CO_2-rich, dew point data collected during this study. The increasing pressure resulted in the increased density and solvent strength of the carbon dioxide. Therefore, the solubility of the compound increased with pressure. An upper limit of solubility in carbon dioxide was evidenced by a steep increase in the slope of the dew point loci, corresponding to an immiscibility gap between the carbon dioxide-rich phase and the DVA-rich or fluorodiol-rich phase. None of the systems exhibited a critical point and three phase equilibria was not observed as the pressure diminished.

The dew point loci of CO_2 with a series of fluorinated diols: 2,2,3,3-Tetrafluoro-1,4-butanediol (TFBD), 2,2,3,3,4,4-Hexafluoro-1,5-pentanediol (HFPD), and 1H,1H,9H,9H-Perfluoro-1,9-nonanediol (PFND) at 323 K are shown in Figure 10. 1H,1H,12H,12H-Perfluoro-1,12-dodecanediol (PFDD) exhibited no detectable solubility (< 0.01 weight percent) in carbon dioxide in the pressure range studied. Binary system phase boundary loci associated with CO_2 and a series of fluorinated or semi-fluorinated compounds containing polar moieties are typically not monotonic. As the number of fluorinated carbons, “n”, increases, the molecular weight of the compound also increases, diminishing the solubility of the compound in carbon dioxide due to entropic effects. However, an increase in “n” also increases the fluorinated segment of the molecule, while retaining the structure of the alcohol end groups. Therefore, an increase in “n” can also increase the carbon dioxide-philic portion of the molecule, thus enhancing its solubility in

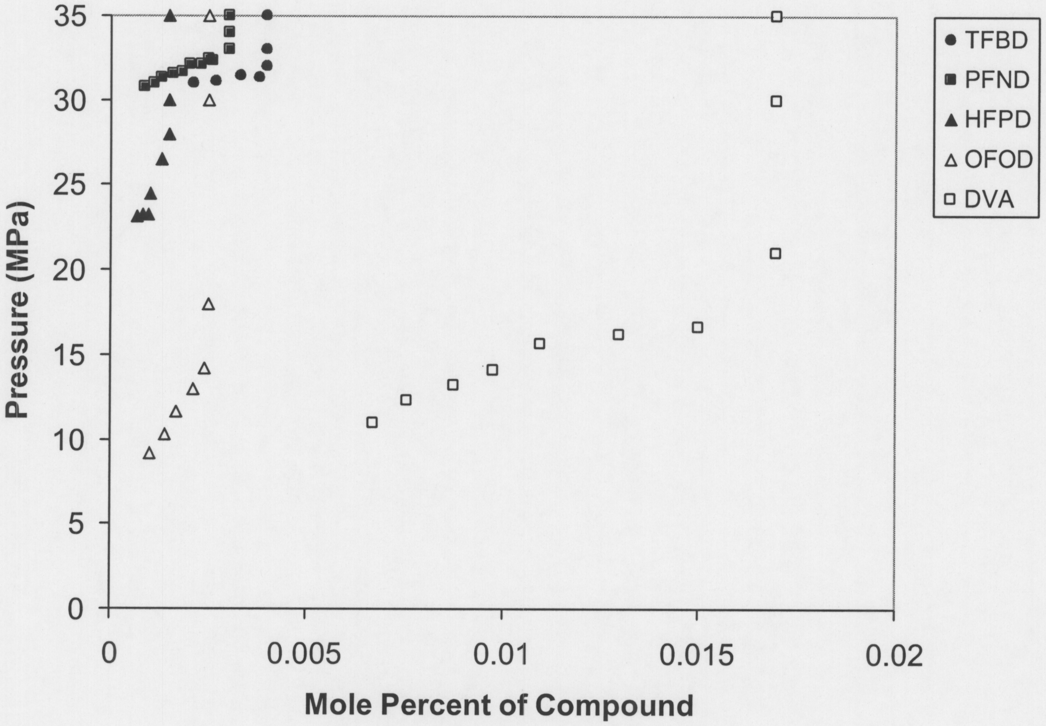


Figure 10 Pressure-composition Diagram of the CO_2-monodisperse Compounds Binary System at 323K.

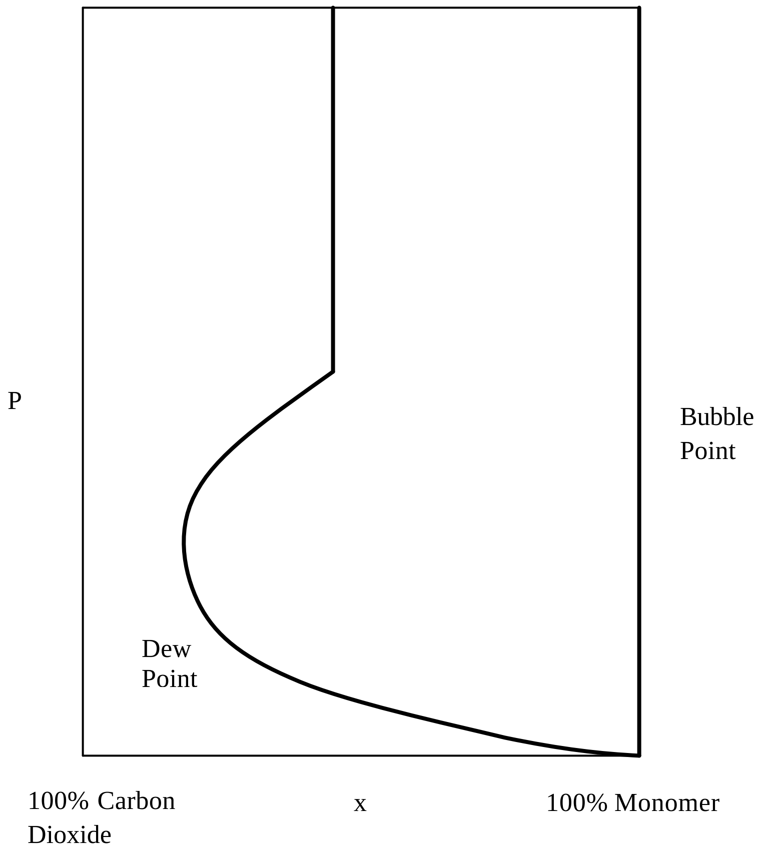


Figure 11 Type 4 Diagram Corresponding to the Dew Point Data Collected During the Phase Behavior Studies

CO_2. Further, the shape of the two-phase boundary illustrated in Figure 11 may change as “n” increases. This can cause dew point loci to cross, resulting in one compound exhibiting a lower dew point pressure than another over a dilute concentration range, but greater miscibility pressures over a more concentrated range of composition. These competing trends can result in an optimal value of “n” for carbon dioxide solubility in specified concentration ranges. For example, Figure 10 shows that HFPD exhibited the greatest carbon dioxide solubility at concentrations of less than 0.3 weight percent or $1.5x10^{-3}$ mole percent. However, the maximum solubility of the fluorinated compound in carbon dioxide, which corresponds to the concentration at which the dew point locus abruptly increases in slope, was lowest for HFPD.

The carbon dioxide solubility of a semi-fluorinated diol, 3,3,4,4,5,5,6,6-Octafluorooctan-1,8-diol (OFOD), was also determined. This compound was more CO_2-soluble than any of the other fluorinated diols studied at concentrations up to 0.75 weight percent or 0.0025 mole percent, as evidenced by the low dew point pressures of this binary mixture (Figure 10). The high degree of CO_2-miscibility of OFOD was attributed to having enough carbons to reduce the polarity of the compound, while at the same time, having a high enough degree of fluorination to increase its CO_2-solubility without any adverse effects due to its elevated molecular weight. Thus, OFOD was soluble at much lower pressures than PFND, which differed in carbon chain length by only one, but had six additional fluorine atoms.

The CO_2-DVA data illustrate that this nonfluorinated organic compound exhibited a significant degree of carbon dioxide solubility relative to the fluorinated diols. A comparison of the CO_2-DVA and CO_2-OFOD dew point loci indicated that at 323 K and pressures below 20 MPa, both compounds exhibited significant miscibility. Therefore, dense carbon dioxide would be a suitable reaction medium for DVA and OFOD at the appropriate temperature and pressure. Significantly higher pressures would be required for mixtures of DVA and any of the other fluorinated diols studied.

6.4 Pseudo-Binary Diagram of the CO_2-(DVA/OFOD) System

Stoichiometric mixtures of DVA and OFOD were combined with carbon dioxide to verify that a single phase solution of these components could be achieved at 323 K and at pressures below 20 MPa. The results were presented in the form of a pseudo-binary diagram (Figure 12). This diagram is a “slice” of an isothermal ternary prism of the CO_2-DVA-OFOD system (a series of isothermal ternary diagrams at increasing pressure), with the sides of the prism corresponding to the P_x diagrams of the binaries (Figure 13). Figure 12 shows that at pressures below 13 MPa, these reactants were miscible with carbon dioxide at concentrations up to a combined mole fraction of $3.5\text{x}10^{-3}$. The miscibility of these two monomers then allowed for a polymerization to be conducted enzymatically in supercritical carbon dioxide. The resulting polyester product had a weight average molecular weight of 8232 Da and a polydispersity of 1.76. Performing the reaction in supercritical carbon dioxide provides the following advantages: 1) elimination of contamination problems caused by the monomers and low molecular weight oligomers, resulting in polymers with low polydispersities and 2) improved control of the reaction exotherm and polymer's viscosity.

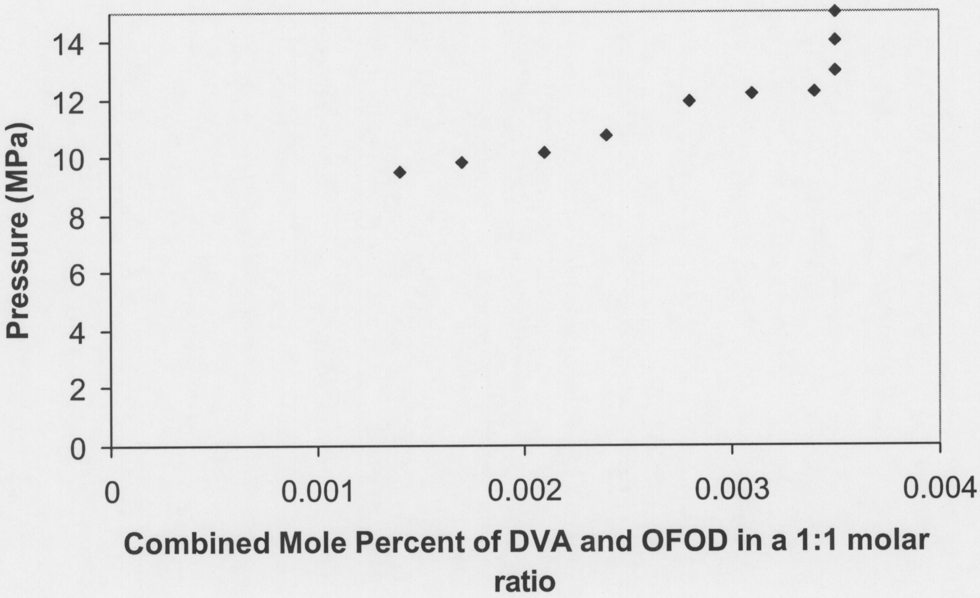


Figure 12 Pressure-composition Diagram of the CO_2-(DVA/OFOD) Pseudo-binary System at 323K

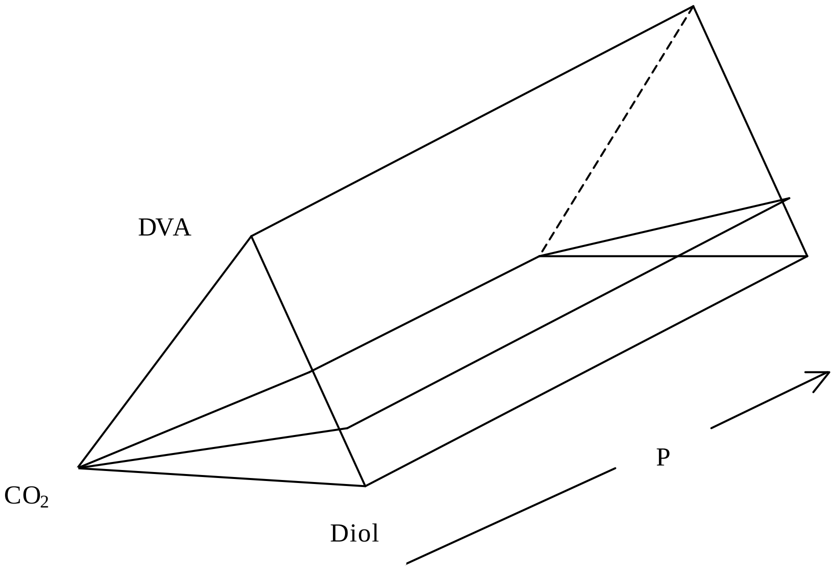


Figure 13 The Isothermal Ternary Prism for the CO_2-(DVA/OFOD) System

6.5 Polymer Data

A pseudo-binary diagram for mixtures of carbon dioxide and a polydisperse fluorinated polyester (average $M_n = 1800$) synthesized from DVA and TFBD is illustrated in Figure 14. This diagram is also characteristic of a Type 4 system. The graph exhibits a relatively flat plateau for polymer mole fractions between 1.0×10^{-6}-1.4×10^{-6} (weight fractions between 1.9×10^{-3} and 2.7×10^{-3}) at 20 MPa. Although less soluble than fluoroacrylate-based and fluoroether-based polymers of comparable molecular weight, this polymer exhibited enough solubility to use supercritical carbon dioxide as a reaction medium for polyester synthesis.

6.6 Conclusion

The phase behavior of divinyl adipate, fluorinated diols of varying chain length, and a resulting polyester product have been investigated. Dew point data collected for the binary systems indicated that TFBD, HFPD, PFND, OFOD, and DVA all exhibited solubility in supercritical carbon dioxide. The most CO_2-soluble compound studied was DVA. Fluorination of the diols, with the exception of PFDD, provided enhanced solubility in supercritical carbon dioxide when compared to their hydrogenated counterparts. While PFND exhibited the highest solubility of the fluorinated compounds on a weight percent basis and TFBD exhibited the highest solubility on a molar basis, OFOD became one phase with carbon dioxide at much lower pressures.

A resulting polyester product synthesized from DVA and TFBD also displayed some degree of miscibility with carbon dioxide. After demonstrating that an equimolar

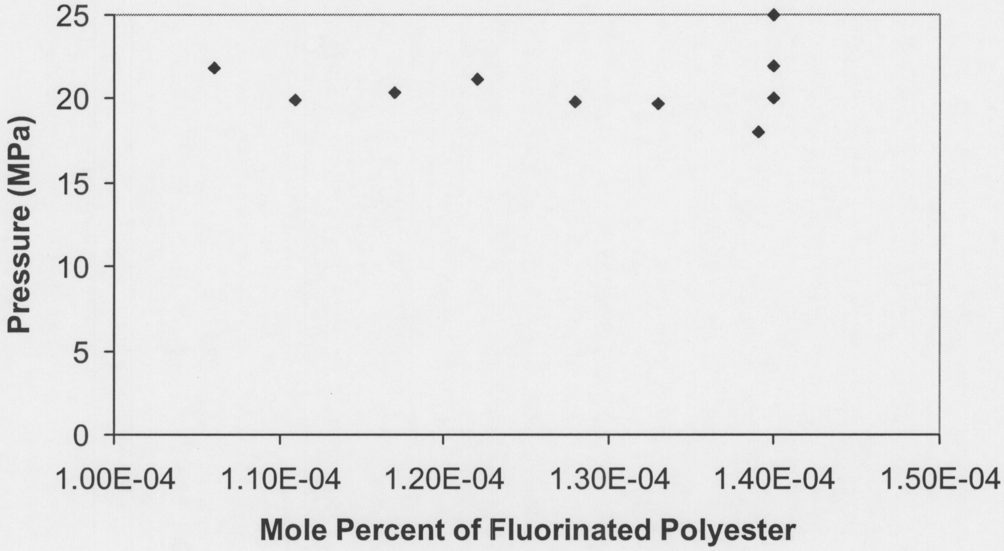


Figure 14 Pressure-composition Diagram of the CO2-fluorinated Polyester Pseudo-binary System at 323K

mixture of DVA and OFOD showed appreciable solubility in the reaction environment, a polymerization was conducted between DVA and OFOD in supercritical carbon dioxide with Novozym® 435 at 50°C (323K) for 24 hours. This reaction demonstrated that it is possible to synthesize a fluorinated polyester of appreciable molecular weight in this medium.

7.0 BIOCATALYTIC SYNTHESIS OF FLUORINATED-HYDROXYLATED POLYESTERS

7.1 Objective and Introduction

The interest in research for polyesters with pendant functional groups has been growing for the past few decades. However, synthesizing these materials chemically can present some challenges. Multiple steps are required and the resulting products can be branched or cross-linked. Using an enzyme to catalyze these reactions results in materials that are linear with pendant functional groups. Recent studies have demonstrated that linear polyesters with pendant hydroxyl groups can be biocatalytically synthesized in one step.(55) The unique specificity of enzymes can extend this research one step further by producing copolymers comprised of fluorinated backbone segments and pendant hydroxyl segments. This product would most likely have interesting properties and could potentially have applicability as a surfactant or compatibilizer.

The synthesis of these materials can be accomplished by using divinyl adipate (DVA), a multi-hydroxyl compound such as glycerol or 1,2,6-trihydroxyhexane (hexanetriol, HT), and a fluorinated diol. By varying the initial ratio of diol to triol, the properties of the resulting polymer can also be altered. Once the materials are synthesized and characterized, they can be tested for what their potential application may have commercially.

The regiospecificity of enzymes allows for these types of reactions to be possible. However, enzymes possess enantioselectivity as well. Therefore, as a side project of this research, 1,2,4-butanetriol was also reacted with divinyl adipate to determine if and what molecular weight polymers can be synthesized using these two monomers and if the reaction was at all enantioselective. If so, it would also be possible to synthesize optically active polyesters using this methodology.

7.2 Procedure

All reactions were performed in Wheaton vials of varying sizes depending on the desired quantity of polymer to be synthesized. An equimolar amount of DVA was reacted with an equimolar amount of a mixture containing a fluorinated diol and a triol. The ratio of diol to triol was varied in order to study its effect on molecular weight and the composition of the final polymer product. After incubating the monomers at 50°C for 30 minutes, enzyme was added to initiate the reaction. The reactions proceeded for 24 hours, after which time the products were isolated and analyzed without further purification via gel permeation chromatography (GPC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF). Hydroxyl number analysis was also performed on each type of polymer synthesized. Depending on the ratio of diol to triol, the final polymer product ranged from an opaque, viscous, liquid polyester to a white, amorphous, solid polyester.

For the reactions in which DVA was reacted with 1,2,4-butanetriol, 3 different enzymes and concentrations were screened in order to determine which was optimal. The reactions were carried out at 50°C for 24 hours. Products were isolated and analyzed via

GPC. DVA was reacted with racemic, R(+) and S(-) 1,2,4-butanetriol and studied in a solvent-free environment.

7.3 Fluorinated-Hydroxylated Polyester Reactions

Reactions were first carried out between divinyl adipate (DVA) and various ratios of OFOD and glycerol in a solvent-free environment at 50°C with Novozym® 435 for 24 hours. The number average molecular weights of these polymers ranged from 1562 Da (90% glycerol, 10% OFOD) to 4764 Da (0% glycerol, 100% OFOD) (Figure 15). However, when hydroxyl number analysis was performed on these polymer samples, the number of OH groups per repeat unit was very low. For example, the polymer sample containing 90% glycerol had only 0.21 OH groups per repeat unit when, ideally, this number should be around 0.90 or greater. Therefore, glycerol was replaced with 1,2,6-trihydroxyhexane (HT) to see if the hydroxyl number improved at all. The effect of replacing glycerol with 1,2,6-trihydroxyhexane on polymer molecular weight can be seen in Figure 16. Hydroxyl number results will be discussed in a later section. The appearance of the final polymer product varied depending on the initial ratio of fluorinated diol to triol. When higher amounts of OFOD were used, the polymers were white, amorphous solids. However, at higher amounts of triol, the polymers were opaque, viscous liquids. When the initial ratio of diol to triol was 1:1, the polymers were still more viscous in nature.

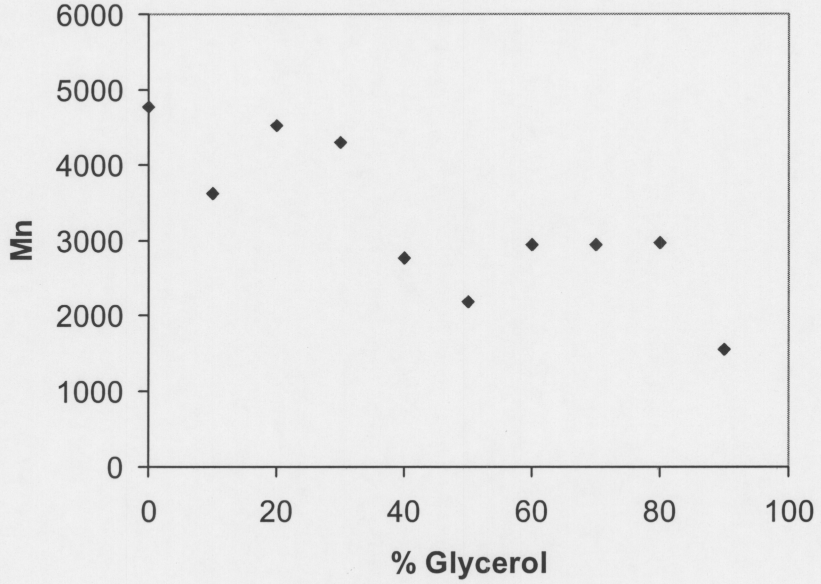


Figure 15 Effect of Glycerol Content on the Molecular Weight of Polyesters Synthesized from DVA and OFOD/Glycerol Mixtures

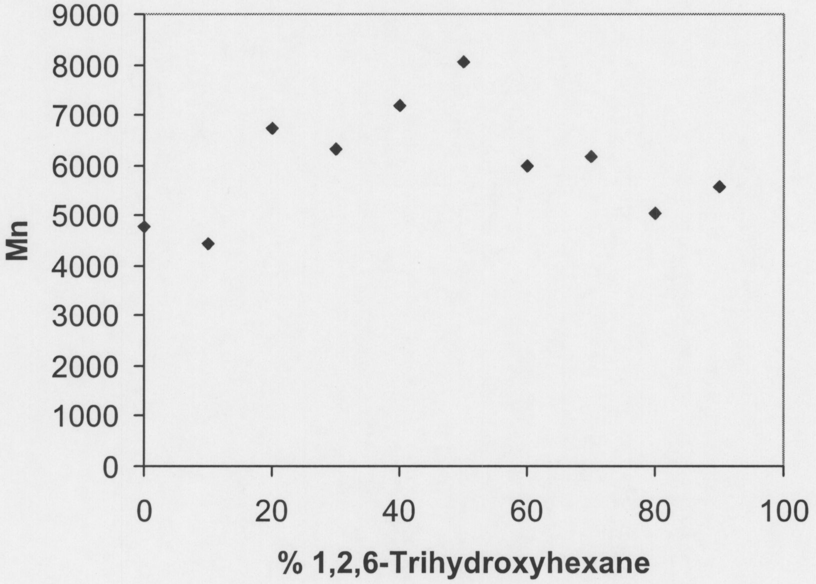


Figure 16 Effect of 1,2,6-Trihydroxyhexane Content on the Molecular Weight of Polyesters Synthesized from DVA and OFOD/1,2,6-Trihydroxyhexane Mixtures

7.4 Characterization of Fluorinated-Hydroxylated Polyesters

7.4.1 Hydroxyl Number

Hydroxyl number analysis was performed on each of the polymers synthesized from DVA and various mixtures of either OFOD and glycerol or OFOD and 1,2,6-trihydroxyhexane. When a linear triol is polymerized with DVA, the number of hydroxyl groups per repeat unit should essentially be one. However, when a diol is polymerized with DVA, this number should be zero, although slight fluctuations may arise depending on the end group functionalities. Previous research has demonstrated this to be the case.(55) Therefore, it should stand to reason that when DVA is polymerized with a mixture of diol and triol, the number of hydroxyl groups per repeat unit should be between zero and one, depending on the diol to triol ratio.

As previously stated, the hydroxyl numbers of the polymers synthesized with glycerol were lower than anticipated, thus prompting the switch from glycerol to 1,2,6-trihydroxyhexane. This led to an increase in the hydroxyl numbers of the resulting polyesters (Figure 17). It was also demonstrated that if OFOD was replaced with HFPD, the same trend in hydroxyl number occurred (Figure 18).

7.4.2 MALDI-TOF Analysis

In addition to hydroxyl number analysis, MALDI-TOF was also used to determine the following properties of the polyesters: 1) the overall percentage of fluorinated segments, 2) the ratio of fluorinated to hydroxylated segments, and 3) the percentage of various chain types in the sample. This technique was accomplished by

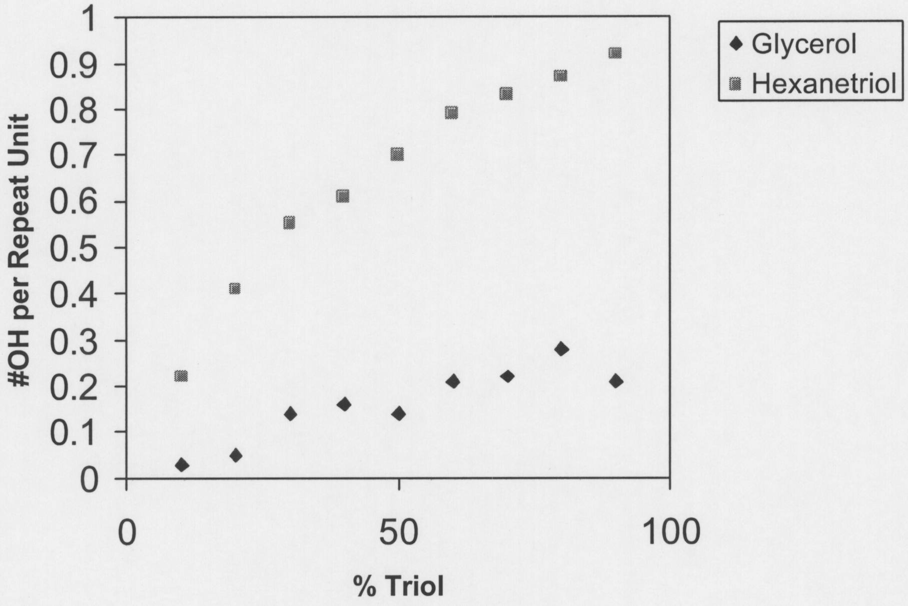


Figure 17 The Number of Hydroxyl Groups per Repeat Unit for Polyesters Synthesized with Glycerol and 1,2,6-Trihydroxyhexane using OFOD as the Fluorinated Diol

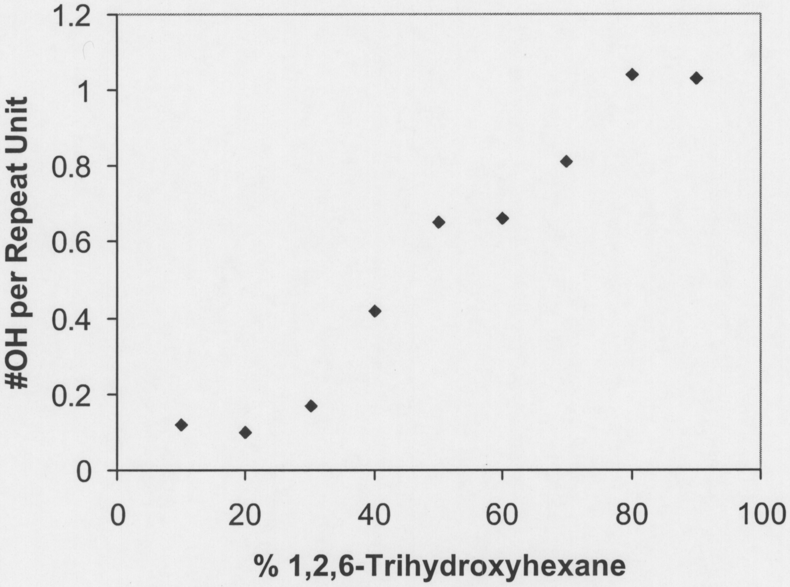


Figure 18 The Number of Hydroxyl Groups per Repeat Unit for Polyesters Synthesized with 1,2,6-Trihydroxyhexane and HFPD

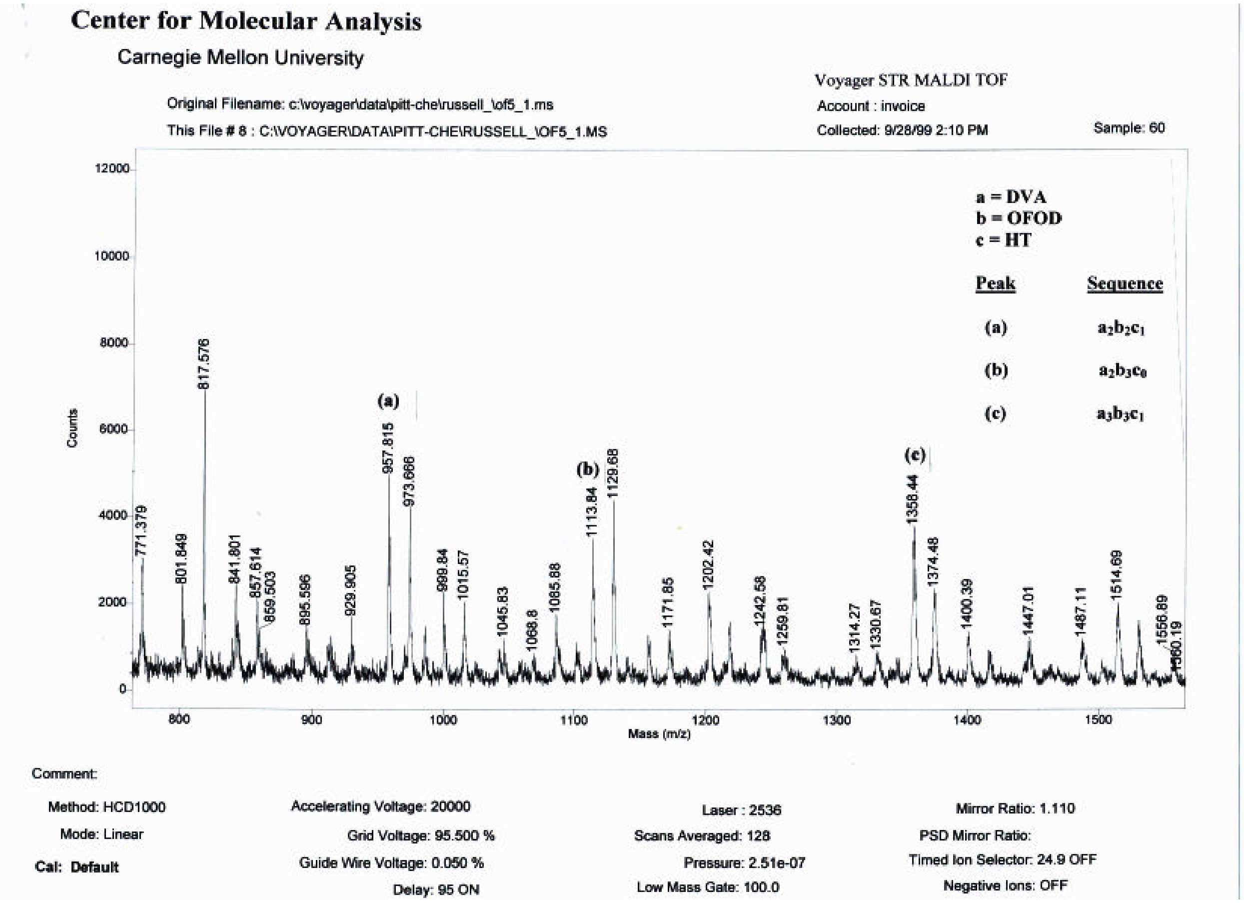
calculating each possible combination of monomers in a given sequence, along with the various types of end groups and verifying that the peak appeared on the MALDI spectra. An example of this method can be seen in Figure 19. Once the peaks were identified, the information was summarized by correlating how the initial ratio of fluorinated diol to triol affected the final properties of the polymer (Table 8).

Table 8 Analysis of MALDI-TOF Spectra of Fluorinated-Hydroxylated Polyesters

Initial Ratio of OFOD:HT	% Fluorinated	Fluorinated/ Hydroxylated Ratio	% of Chains that are X% Fluorinated		
			X = 33	X = 50	X = 100
9:1	86%	6.3	1.7%	0%	54%
5:1	69%	2.2	4.0%	7.0%	26%
2:1	65%	1.8	4.8%	15%	22%
1.5:1	55%	1.2	21%	15%	15%
1.25:1	53%	1.1	19%	13%	15%

Reaction conditions: Shaker temperature = 50°C; Reaction time = 24 hours; Enzyme concentration = 12% (w/w).

The results of Table 8 can also be displayed graphically, once again demonstrating how the initial ratio of fluorinated diol to triol can dictate the composition of the final product. As the initial concentration of OFOD increases, so does the percentage of fluorination of the polymer (Figure 20) and the fluorinated to hydroxylated ratio (Figure 21).



Center for Molecular Analysis

Carnegie Mellon University

Original Filename: c:\voyager\data\pitt-che\russell_\of5_1.ms

This File # 8 : C:\VOYAGER\DATA\PITT-CHE\RUSSELL_\OF5_1.MS

Voyager STR MALDI TOF

Account : invoice

Collected: 9/28/99 2:10 PM

Sample: 60

a = DVA
b = OFOD
c = HT

Peak	Sequence
(a)	$a_2b_2c_1$
(b)	$a_2b_3c_0$
(c)	$a_3b_3c_1$

Comment:

Method: HCD1000

Mode: Linear

Cal: Default

Accelerating Voltage: 20000

Grid Voltage: 95.500 %

Guide Wire Voltage: 0.050 %

Delay: 95 ON

Laser : 2536

Scans Averaged: 128

Pressure: 2.51e-07

Low Mass Gate: 100.0

Mirror Ratio: 1.110

PSD Mirror Ratio:

Timed Ion Selector: 24.9 OFF

Negative Ions: OFF

Figure 19 MALDI-TOF Spectra of a Fluorinated-Hydroxylated Polyester

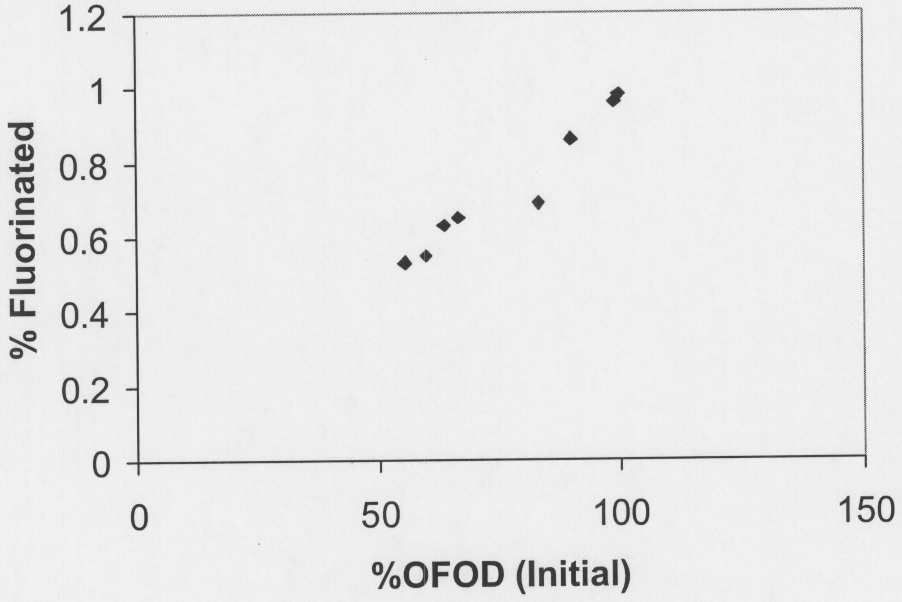


Figure 20 Effect of Initial Concentration of OFOD on Percent Fluorination of the Polyester

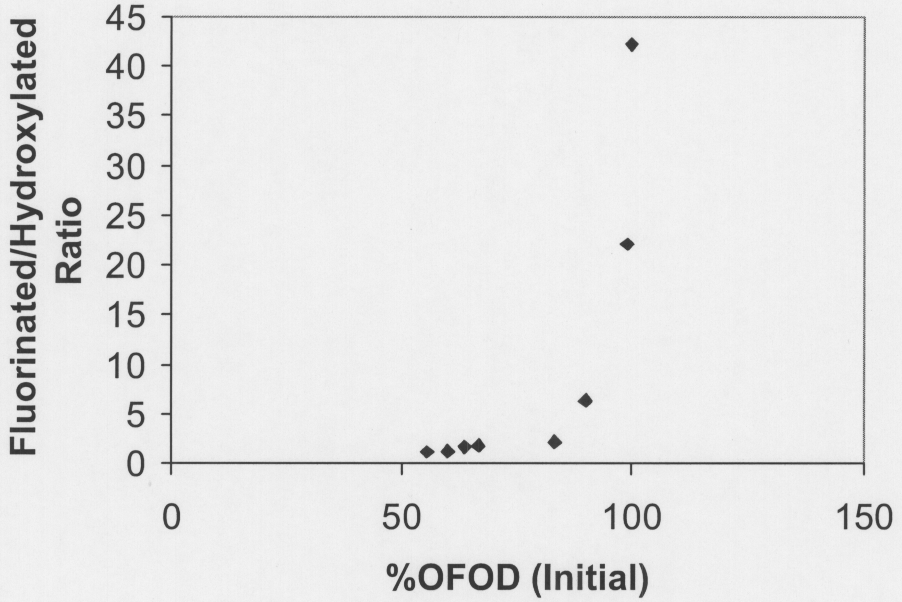


Figure 21 Effect of Initial Concentration of OFOD on the Fluorinated/Hydroxylated Ratio of the Polyester

7.5 Reactions with DVA and 1,2,4-Butanetriol

In addition to studying glycerol and 1,2,6-trihydroxyhexane, 1,2,4-butanetriol was also reacted with DVA in order to assess whether or not an optically active polyester could be produced enzymatically. The first set of experiments involved reacting racemic 1,2,4-butanetriol with DVA in a solvent-free environment at 50°C for 24 hours. An enzyme screen demonstrated that three lipases (Novozym® 435, *Thermomyces sp.* (L8), and *Mucor miehei* (L9)) produced polyester, although 2% (w/w) Novozym® 435 was clearly the best enzyme for the reaction (Figure 22). DVA was also reacted with enantiomerically pure forms of 1,2,4-butanetriol with two different concentrations of Novozym® 435 under the same reaction conditions. As indicated in Figure 23, the higher molecular weight polyesters were produced using the S(-) form, suggesting that the enzyme favors this enantiomer over the R(+) form.

Attempts were made to further study the enantioselectivity of Novozym® 435 using a Perkin Elmer 241 Polarimeter to measure angles of rotation. However, this apparatus was operating incorrectly and although several efforts were made, it was not properly fixed during the course of this research.

7.6 Conclusion

The synthesis of polyesters containing both fluorinated and hydroxylated segments was accomplished. After concluding that glycerol did not produce polyesters with sufficient hydroxyl numbers, it was replaced with 1,2,6-trihydroxyhexane. Once the

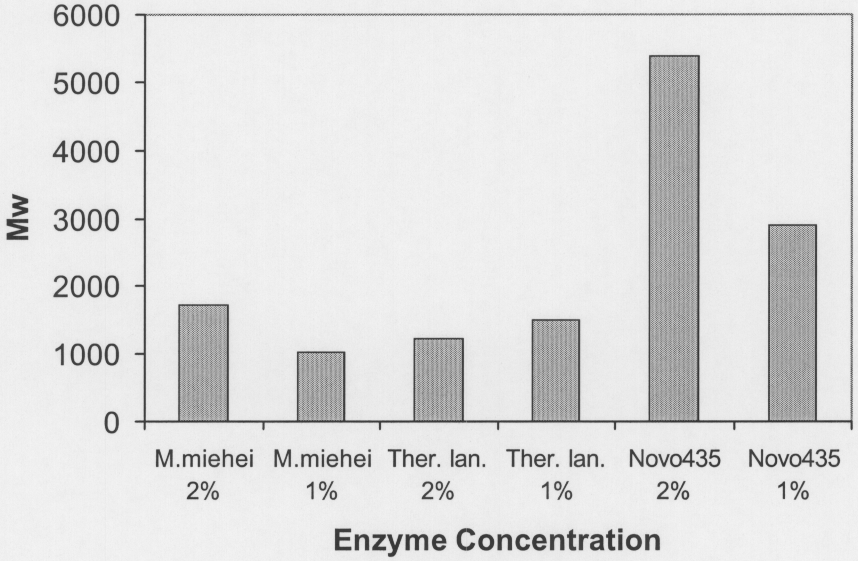


Figure 22 Effect of Enzyme Type and Concentration on the Reaction between DVA and Racemic 1,2,4-Butanetriol

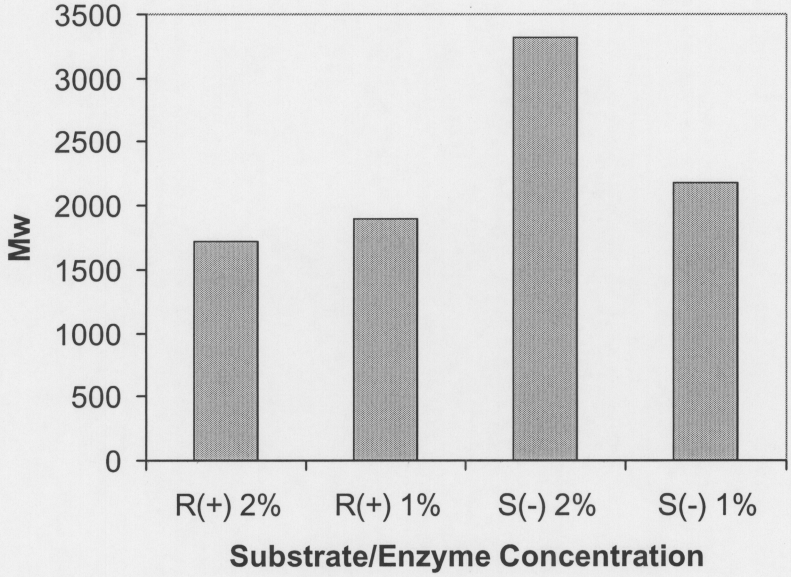


Figure 23 Molecular Weight Comparison of Polyesters Synthesized from DVA and Either R(+) or S(-) 1,2,4-Butanetriol

molecular weights of these materials were determined, hydroxyl number analysis demonstrated that as the initial concentration of triol increased, so did the number of hydroxyl groups per repeat unit. MALDI-TOF analysis was able to verify the composition of the final polyester product, such as the percentage that was fluorinated, the fluorinated to hydroxylated ratio, and the percentage of various chain types in the product. As a side project, DVA was also reacted with 1,2,4-butanetriol to assess the feasibility of producing an optically active polyester enzymatically.

8.0 ENZYME BEHAVIOR IN IONIC LIQUIDS

8.1 Objective and Introduction

An area of research that has garnered quite an amount of attention of late is ionic liquids. Ionic liquids are room temperature liquids that are comprised entirely of ions. They are thermally stable, remain liquid over a broad temperature range, and can dissolve a variety of compounds. What has been the greatest interest to researchers is that they have no measurable vapor pressure, thus eliminating the emission of toxic vapors to the environment.(19-21) Ionic liquids are typically comprised of small, negatively charged ions (anions) and large, bulky, positively charged ions (cations). The ionic liquid used for this research was 1-butyl-3-methylimidazolium hexafluorophosphate (Figure 24). In an effort to combine a green solvent with a green catalyst, it was important to determine whether or not enzymatic catalysis in this type of environment was possible.

This hypothesis was proven to be correct in our laboratory by conducting the thermolysin-catalyzed synthesis of *Z*-aspartame from carbobenzoxy-*L*-aspartate and *L*-phenylalanine methyl ester hydrochloride in the ionic liquid.(131) This particular ionic liquid was selected because it is stable in the presence of both air and water.(127) It was found that the rate of the reaction in the ionic liquid was competitive with that in organic solvents. Also, the enzyme was found to be recyclable and highly stable in this media.

Therefore, because biocatalysis in an ionic liquid was successful, this line of research was extended in order to determine the stability of other enzymes in this

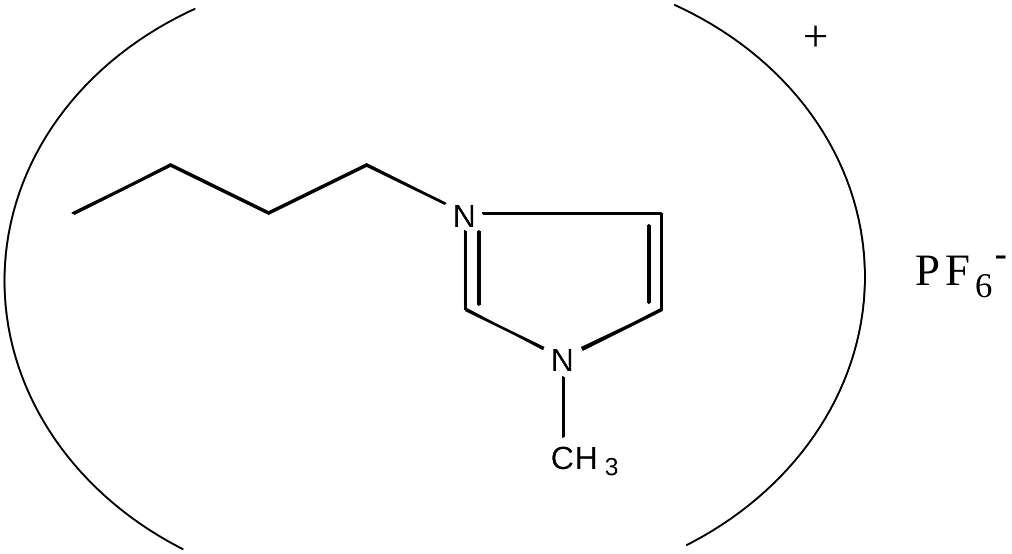


Figure 24 The structure of the Ionic Liquid (1-butyl-3-methylimidazolium hexafluorophosphate)

environment, as well as monomer solubility. More specifically, to examine the stability of lipases, such as Novozym® 435 and porcine pancreatic lipase, both with and without the presence of substrates at 50°C for various time periods. If monomers of interest, such as divinyl adipate and 1,4-butanediol exhibit appreciable solubility, then the polytransesterification of these two monomers can be performed and studied in the ionic liquid (Figure 25).

8.2 Procedure

The stability of the enzymes Novozym® 435 and porcine pancreatic lipase was studied both in the ionic liquid and a traditional organic solvent, tetrahydrofuran. Approximately 10mg of enzyme were incubated for given amounts of time up to 48 hours in either the ionic liquid or the organic solvent at 50°C. The solvent was then removed and the enzyme was assayed for activity using a standard Teitz assay kit. These studies were conducted with and without the presence of the monomers divinyl adipate and 1,4-butanediol. The experiments were repeated in order to verify whether or not they were reproducible. Additionally, the stability of Novozym® 435 was examined in more detail in the time period of 0-50 minutes and the stability of both enzymes was carried out for longer time periods of up to 2 weeks. A high temperature stability study of Novozym® 435 and porcine pancreatic lipase at 70°C was then performed in the ionic liquid and compared to that in octane.

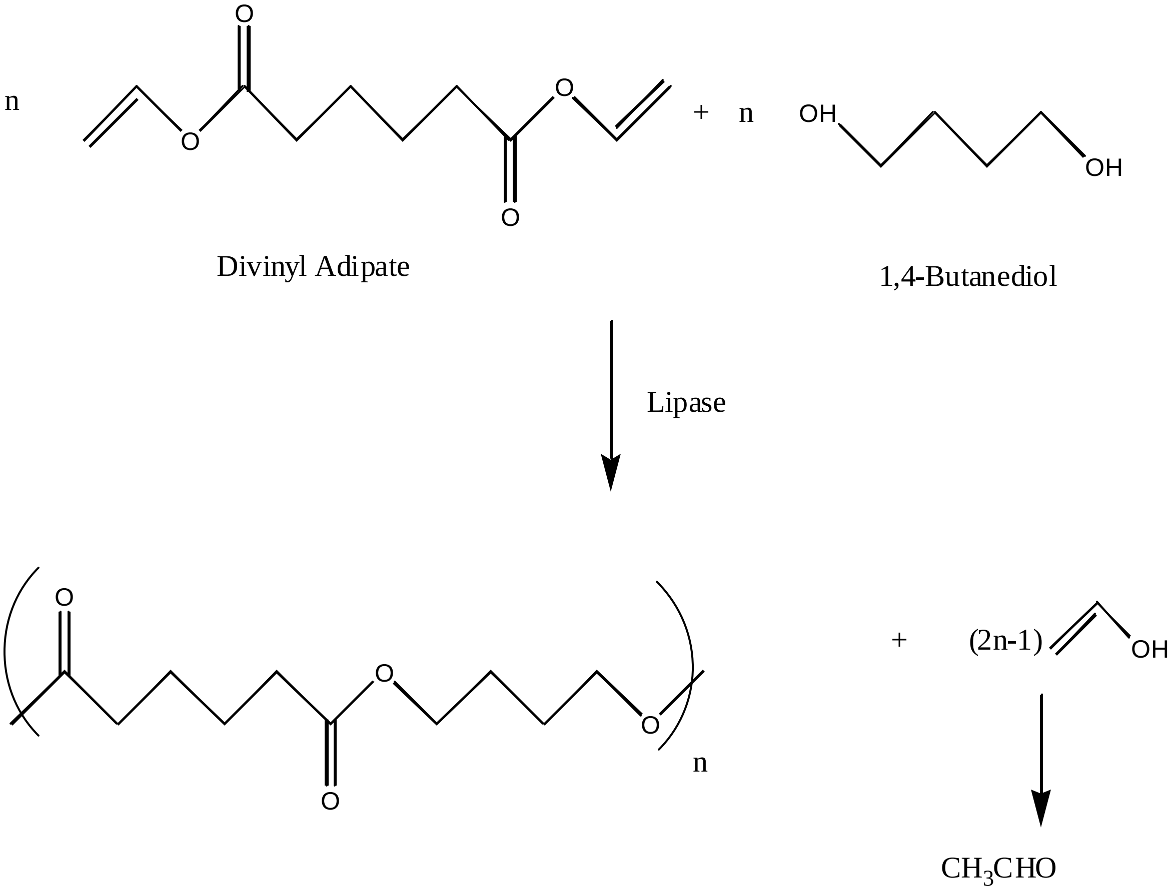


Figure 25 Schematic of a Lipase-Catalyzed Polymerization between Divinyl Adipate and 1,4-Butanediol

The solubility of various monomers of interest was also examined in the ionic liquid. The compounds divinyl adipate (DVA), 1,4-butanediol (BD), 2,2,3,3-tetrafluoro-1,4-butanediol (TFBD), 2,2,3,3,4,4-hexafluoro-1,5-pentanediol (HFPD), and 3,3,4,4,5,5,6,6,-octafluorooctan-1,8-diol (OFOD) were each tested. Polymerization reactions were then run between DVA and those diols that were soluble using the ionic liquid as a solvent. The monomers were first incubated for 30 minutes, after which time the enzyme was added and the reactions were allowed to proceed for 24 hours at 50°C. At the end of the reaction time, when the vials were removed from the incubator, it was noted that the polymer product had precipitated out of the ionic liquid. The precipitated polymers were then isolated and analyzed via gel permeation chromatography.

8.3 Enzyme Stability Studies

The stability of two lipases, Novozym® 435, an immobilized lipase from *Candida antarctica*, and porcine pancreatic lipase was tested in the ionic liquid and compared to that in tetrahydrofuran (THF) at 50°C. The results of these studies can be seen in Figures 26 and 27. After a time period of 48 hours, Novozym® 435 retains approximately 67% of its original activity and porcine pancreatic retains 94%. This is a substantial improvement over activity retentions in THF of 21% and 61%, respectively. In fact, when Novozym® 435 was examined in the time period of 0 to 50 minutes, the enzyme lost no activity when placed in the ionic liquid environment (Figure 28).

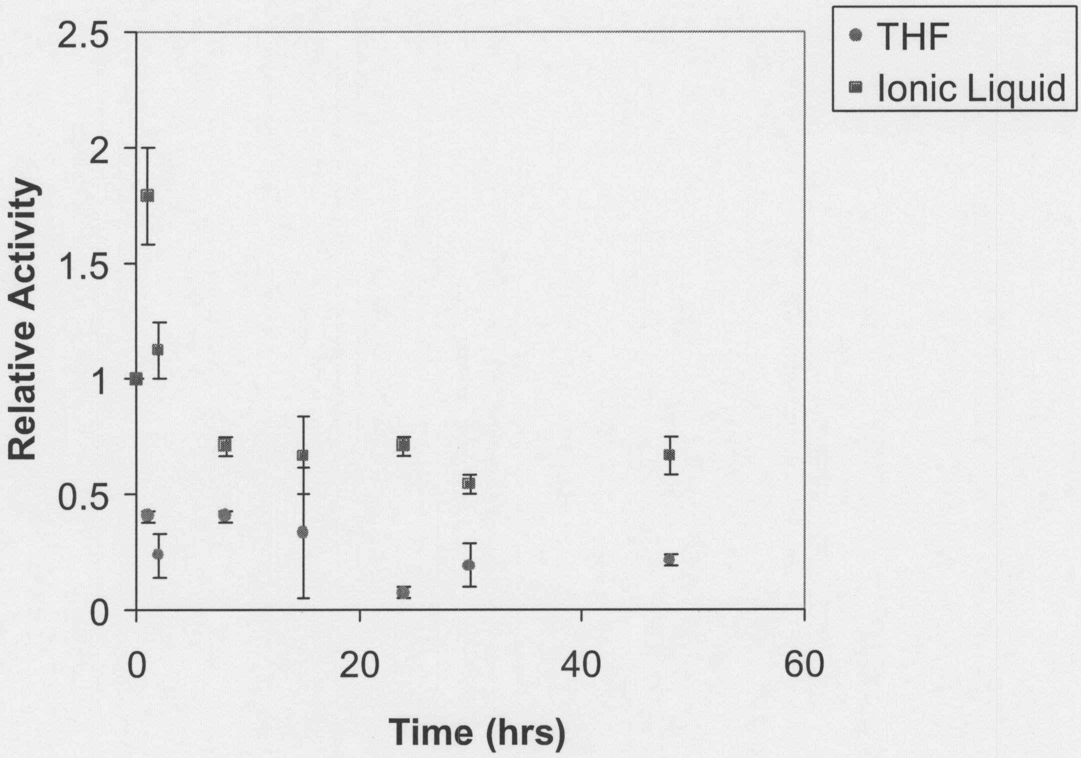


Figure 26 Stability of Novozym® 435 in THF vs. Ionic Liquid at 50°C

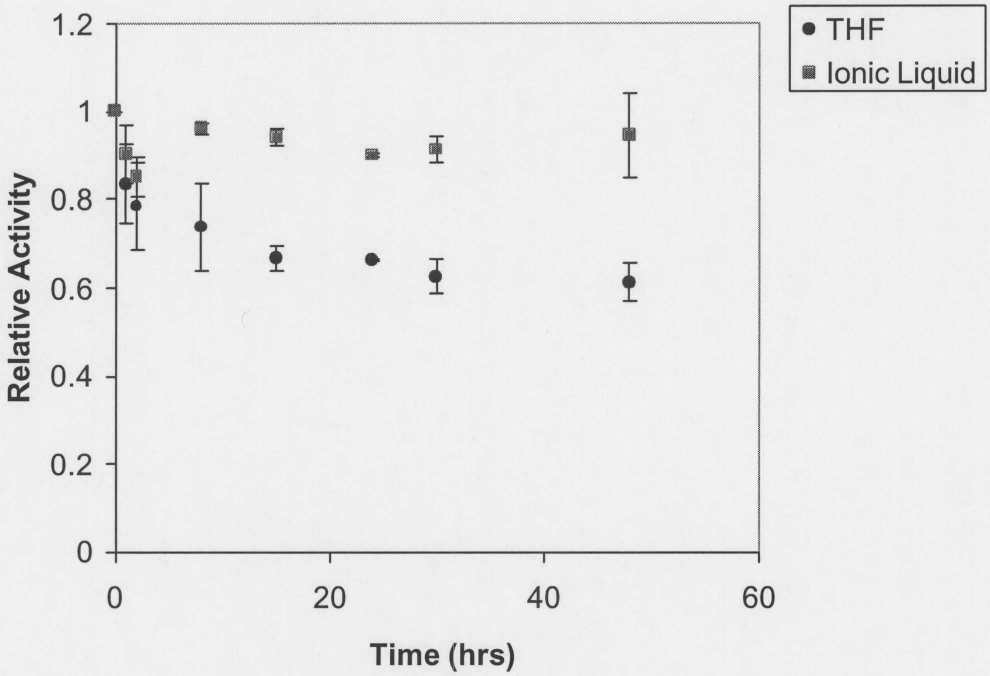


Figure 27 Stability of Porcine Pancreatic Lipase in THF vs. Ionic Liquid at 50°C

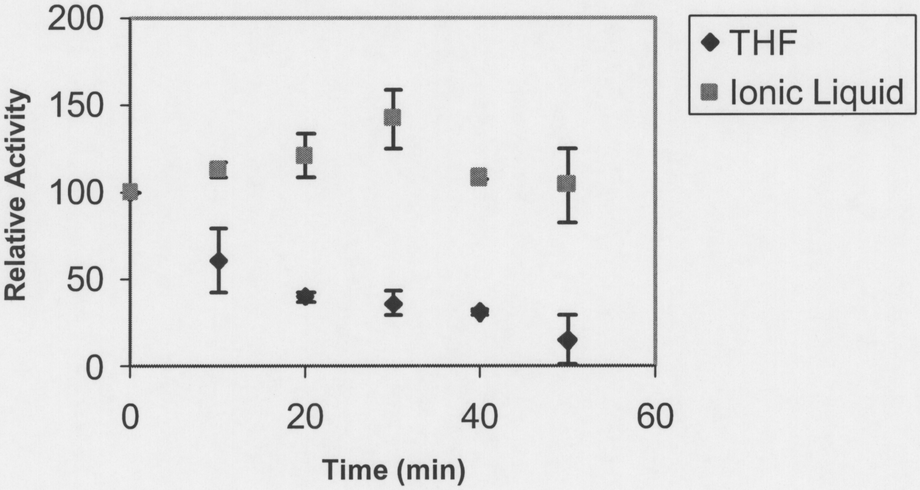


Figure 28 Stability of Novozym® 435 from 0-50 Minutes in THF vs. Ionic Liquid at 50°C

When these experiments were extended to a time period of two weeks, both enzymes still exhibited appreciable stability (Figures 29 and 30). The stability of the enzymes was also tested at a higher temperature, 70°C, and compared to that in octane. Figure 31 demonstrates that even at elevated temperatures, ionic liquids provide a stabilizing environment for the enzymes, superior to that in an organic solvent.

Because the ultimate goal was to use the ionic liquid as a reaction medium in enzymatic catalysis, it was of utmost importance to examine the stability of the enzyme in the ionic liquid in the presence of substrates. Therefore, the experiments were carried out with Novozym® 435 and porcine pancreatic lipase with the monomers divinyl adipate and 1,4-butanediol present for given periods of time in both the ionic liquid and tetrahydrofuran. Previous research had indicated that 1,4-butanediol has a detrimental effect on the enzyme.(145) Figures 32 and 33 illustrate that the ionic liquid stabilizes both enzymes, even when they are in the presence of a hydrophilic substrate like 1,4-butanediol.

8.4 Polymerization Studies

After determining that both Novozym® 435 and porcine pancreatic lipase were highly stable in the ionic liquid environment, the next step was to attempt a polymerization in this medium. The solubility of several monomers was assessed in the ionic liquid (Table 9).

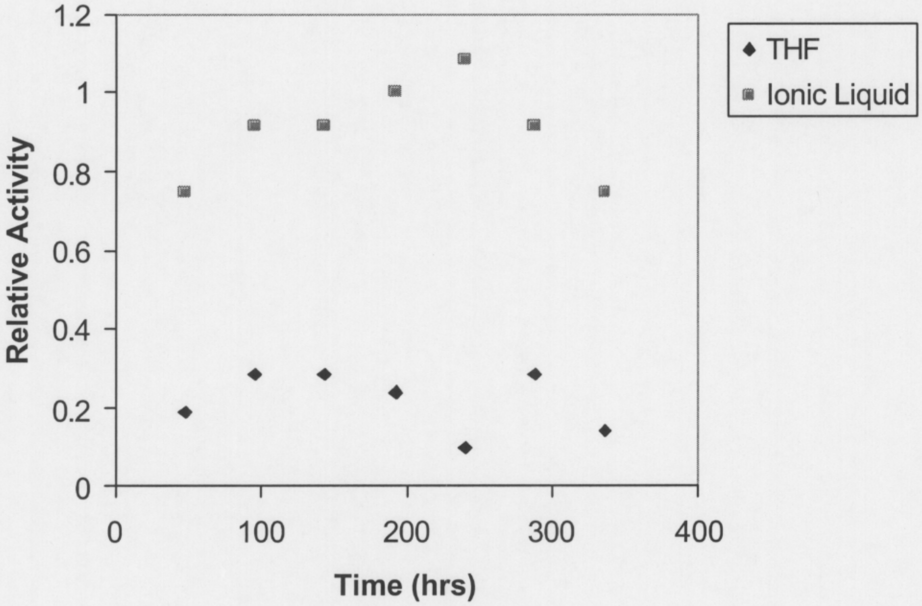


Figure 29 Stability of Novozym® 435 at 50°C for Longer Time Periods (THF vs. Ionic Liquid)

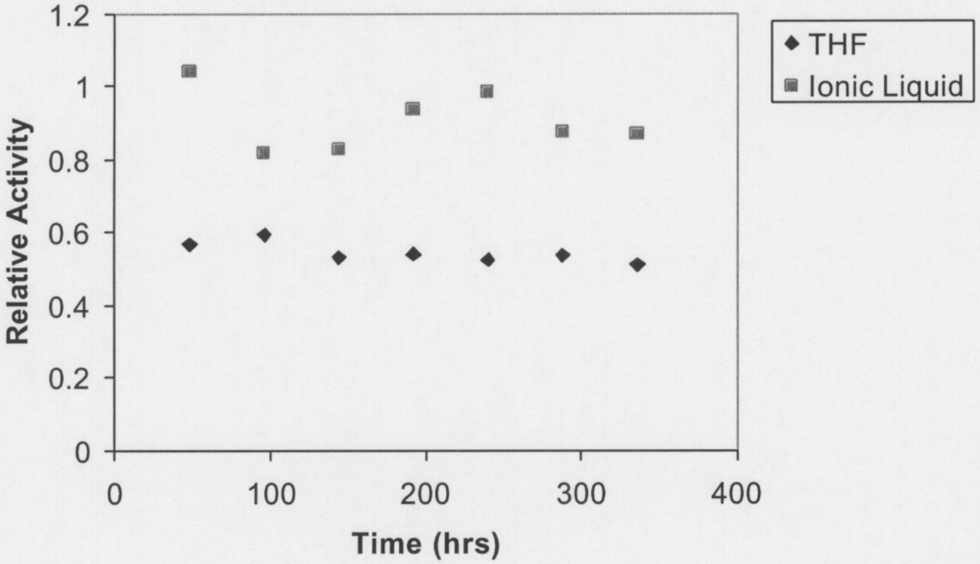


Figure 30 Stability of Porcine Pancreatic Lipase at 50°C for Longer Time Periods (THF vs. Ionic Liquid)

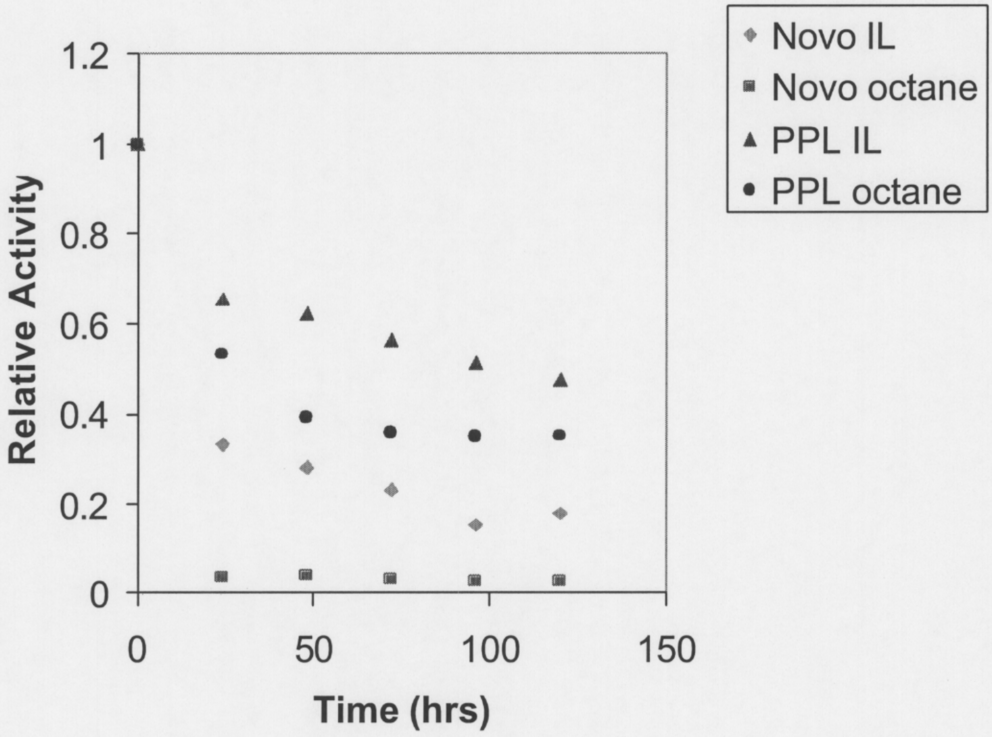


Figure 31 Enzyme Stability at 70°C in Octane vs. Ionic Liquid

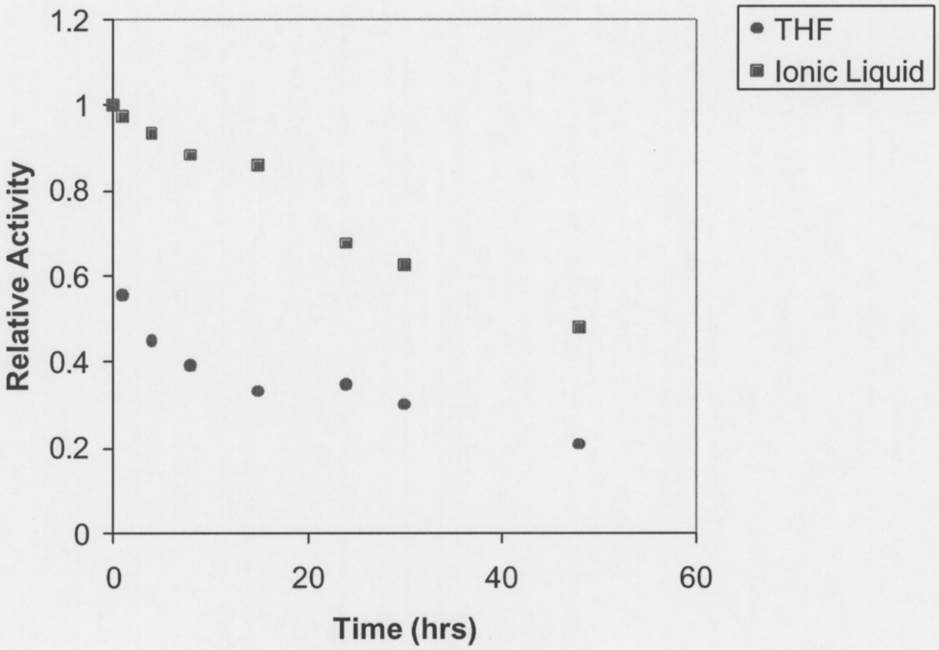


Figure 32 Stability of Novozym® 435 at 50°C in the Presence of Divinyl Adipate and 1,4-Butanediol (THF vs. Ionic Liquid)

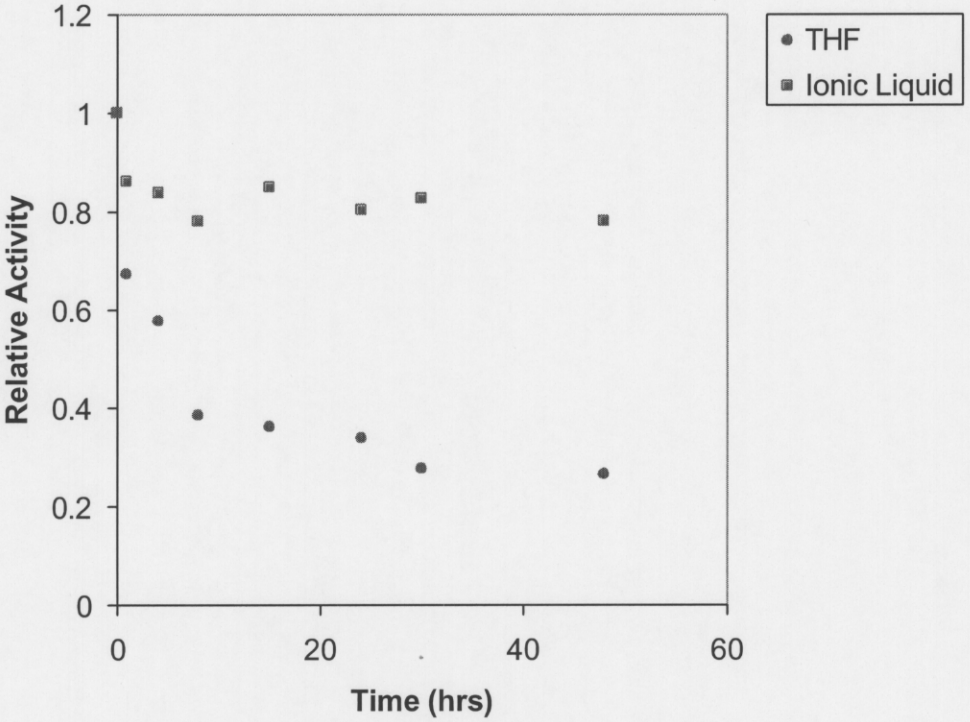


Figure 33 Stability of Porcine Pancreatic Lipase at 50°C in the Presence of Divinyl Adipate and 1,4-Butanediol (THF vs. Ionic Liquid)

Table 9 Monomer Solubility Studies at 50°C in 1-butyl-3-methylimidazolium hexafluorophosphate

Monomer	Soluble (Y/N)?	Concentration
Divinyl Adipate (DVA)	Yes	3.45M
1,4-butanediol (BD)	Yes	0.98M
2,2,3,3-tetrafluoro-1,4-butanediol (TFBD)	No	N/A
2,2,3,3,4,4-hexafluoro-1,5-pentanediol (HFPD)	Yes	0.20M
3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol (OFOD)	Yes	2.23M

After determining that each of the monomers, with the exception of TFBD, demonstrated solubility in this medium, polymerizations were carried out between DVA and each of the soluble diols. The reactions were carried out for 24 hours at 50°C, after which time it was noted that, in each case, the growing polymer had precipitated out of the ionic liquid. The highest molecular weight obtained was from the reaction between DVA and BD (M_w = 2887 Da; PDI = 1.20). The reactions between DVA and HFPD and DVA and OFOD only produced molecular weights of 1254 Da and 962 Da, respectively. An enzyme screen was performed for the DVA + BD reaction. The lipases *Thermomyces sp.* (L8) and *Mucor miehei* (L9) produced polymers with comparable, but still lower, molecular weights than that of Novozym® 435. All other lipases tested did not produce polymer from this reaction.

8.5 Conclusion

The stability of two enzymes, Novozym® 435 and porcine pancreatic lipase (PPL), was examined in the ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, and compared to the stability in a traditional organic solvent, tetrahydrofuran (THF). In both cases, enzyme stability was higher in the ionic liquid than in THF, even up to a time period of two weeks. This was true even when the enzymes were exposed to the monomers divinyl adipate and 1,4-butanediol. Because Novozym® 435 was highly stable in the ionic liquid, biocatalytic polymerizations between divinyl adipate, 1,4-butanediol and two fluorinated diols were conducted. However, the highest molecular weight polyester formed was limited to 2887 Da due to the fact that the growing polymer had precipitated out of the ionic liquid.

9.0 USE OF CARBON DIOXIDE AS A VISCOSITY MODIFIER TO IMPROVE POLYMER MOLECULAR WEIGHT

9.1 Objective and Introduction

Although supercritical carbon dioxide has been explored as a replacement for conventional organic solvents, it has other functions as well. One such example is employing carbon dioxide as a means of reducing the viscosity of polymers. This phenomena has been attributed to the ability of carbon dioxide to absorb between the polymer chains, thus reducing chain entanglement and increasing the free volume, and its function as a molecular lubricant. By combining these effects, the result is a reduction in the viscosity of the polymer solution.(17)

Previous research has investigated the role of diffusion in biocatalytic polytransesterification.(141) It was found that in the solvent-free lipase-catalyzed reaction of divinyl adipate and 1,4-butanediol, diffusion influenced the evolution of polymer molecular weight and polydispersity. Specifically, in the molecular weight range of 3,000-5,000 Da, the viscosity of the polymer solution increased dramatically, thus imposing mass transfer limitations on the system. At molecular weights above 5,000 Da, the mass transfer issues become even more significant, due to the entanglement of polymer chains. Therefore, because carbon dioxide is known to reduce the viscosity of polymer solutions, it was hypothesized that the introduction of carbon dioxide into the reaction system could help to alleviate any mass transfer limitations present at that point of the reaction and increase polymer molecular weight.

If this idea is to be implemented into the current experimental procedure, reactions in which carbon dioxide is present need to be compared with those performed under solvent-free conditions in order to determine if there is any change in polymer molecular weight. Additionally, the effect of carbon dioxide on the enzyme should be examined in great detail. The set of data generated by these experiments can then help to determine whether or not this option is a feasible one for reducing viscosity and improving polyester molecular weight.

9.2 Procedure

Equimolar amounts of divinyl adipate and 1,4-butanediol were placed in the reactor scheme discussed in Chapter 4. After adding the enzyme, the reactor was then sealed. Carbon dioxide was pressurized and allowed to enter the reactor at the desired pressure. The reaction was then carried out at 50°C for various amounts of time. After the reaction was completed, the carbon dioxide was slowly vented and the polymer was collected, isolated and analyzed via gel permeation chromatography (GPC). Control experiments were also performed in the reactor without the presence of carbon dioxide and in the presence of nitrogen in order to study the effects of direct pressure.

Another important aspect of this research was to examine what effect, if any, the carbon dioxide and/or pressure had on the enzyme. The enzyme used in these studies was Novozym® 435, a lipase from *Candida antarctica* immobilized onto a macroporous acrylic resin. In this case, carbon dioxide and/or pressure could affect the enzyme directly or the resin that it is immobilized to.

Therefore, stability studies were conducted on Novozym® 435 in the presence of divinyl adipate, 1,4-butanediol and 1250 psi carbon dioxide at 50°C. The enzyme and substrates were sealed in the reactor for various amounts of time and then assayed using a standard Teitz assay kit. The reactor data was then compared with data obtained from performing stability experiments of the enzyme in a bulk environment at 50°C. Additionally, stability studies were also conducted with Novozym® 435 in the presence of divinyl adipate, 1,4-butanediol and 1250 psi nitrogen in order to assess the effect of pressure itself. These stability studies were then repeated in the same manner in order to verify that the results were reproducible.

Once all stability data was generated, samples of Novozym® 435 were then exposed to both 1250 psi carbon dioxide and nitrogen for various amounts of time, collected and examined using scanning electron microscopy (SEM). The SEM experiments were performed in the Department of Cell Biology and Physiology at the University of Pittsburgh. This was done in order to investigate whether the porous structure of the resin was changing with increased time exposure to carbon dioxide and/or nitrogen. The SEM results were then supplemented with BET analysis, which can determine if there is an overall decrease in available bead surface area over time. Once again, samples of Novozym® 435 that had been exposed to carbon dioxide and nitrogen were collected over various periods of time. Two samples were produced for each time point. The samples were then packaged and sent to Materials Testing Laboratory (Pennington, NJ) for analysis.

9.3 Carbon Dioxide Reactions

After the high pressure reactor, as described in Chapter 4, was assembled and tested for leaks, reactions were carried between divinyl adipate (DVA) and 1,4-butanediol (BD) in the presence of supercritical carbon dioxide at 1250 psi. This pressure was first examined because it places carbon dioxide at supercritical conditions without allowing the monomers to be soluble in it. Other pressures were tested in this range. However, 1250 psi clearly produced polyesters with the highest molecular weights. The molecular weights of the resulting polyesters were compared to those synthesized under solvent-free conditions. The initial reactions were carried out at 50°C for 1 hour and 24 hour time periods. As shown in Figure 34, the 1 hour reactions resulted in producing a polyester with a higher molecular weight when carbon dioxide was present. However, when the reactions were run for 24 hours, carbon dioxide appeared to hinder polymer molecular weight. In order to determine if longer exposure to carbon dioxide impeded molecular weight, reactions were then performed at times of 15 minutes and 30 minutes. During these shorter periods of time, the presence of carbon dioxide had a positive effect on polyester molecular weight (Figure 35), with the most pronounced difference occurring at 30 minutes. Therefore, the 30 minute reaction was run seven more times in order to confirm the reproducibility of the results (Figure 36).

It became quite clear that the effect of supercritical carbon dioxide on the polymerization was positive or negative depending on the reaction time. The longer the reaction was allowed to proceed, carbon dioxide became an increasing hindrance to the reaction.

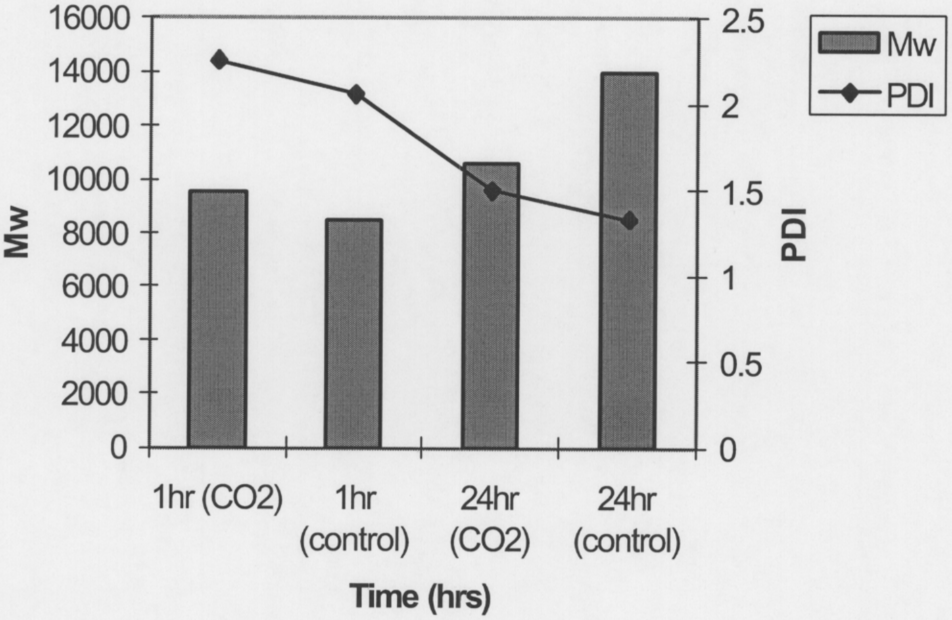


Figure 34 The Molecular Weight Dependence of Polyesters Synthesized with and without Supercritical Carbon Dioxide for Reaction Times of 1 Hour and 24 Hours

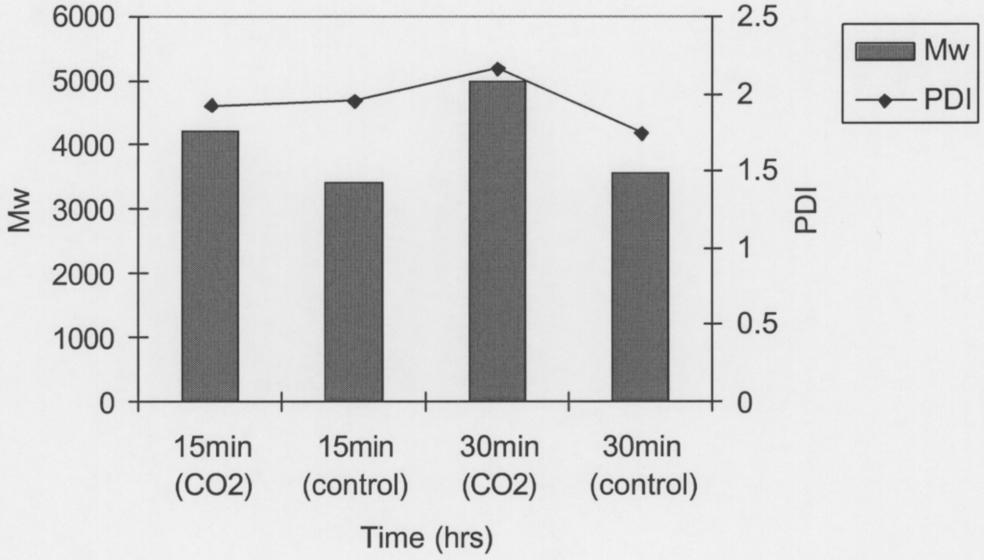


Figure 35 The Molecular Weight Dependence of Polyesters Synthesized with and without Supercritical Carbon Dioxide for Reaction Times of 15 Minutes and 30 Minutes

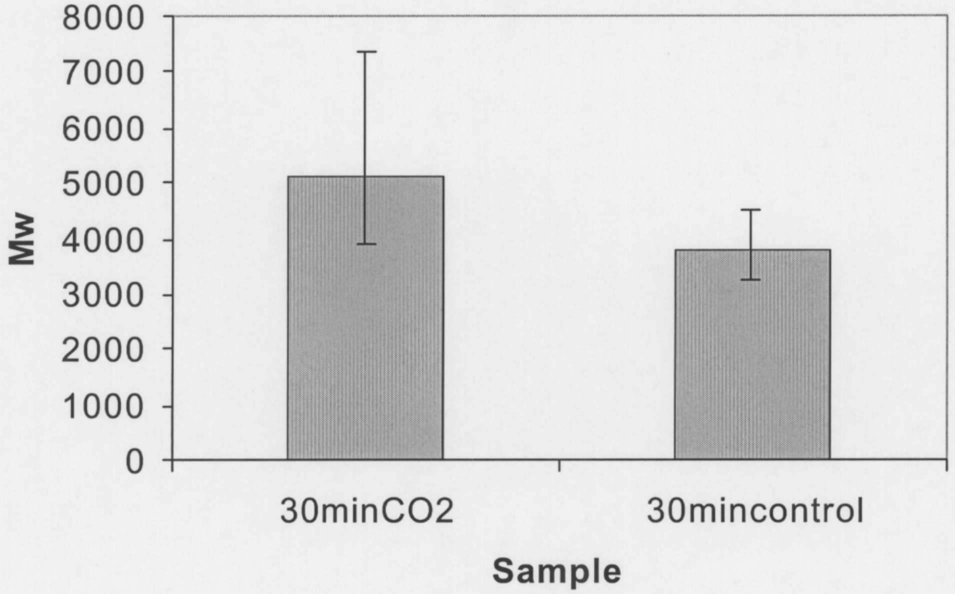


Figure 36 The Reproducibility of Polymer Molecular Weight at a Reaction Time of 30 Minutes

At reaction times below 1 hour, polyester molecular weight is higher in the reactions where carbon dioxide is added. However, this trend begins to reverse itself at times above 1 hour. At 24 hours, the molecular weight of polyesters synthesized under bulk conditions exceeds that of the reactions performed in the presence of supercritical carbon dioxide. It was very clear that, while at first a positive effect, prolonged exposure of the reaction to carbon dioxide hindered overall productivity. A potential hypothesis for this observed phenomena was that carbon dioxide and/or pressure had a detrimental effect on the enzyme itself. The studies performed to examine this hypothesis will be discussed in a later section.

9.4 Nitrogen Reactions

Another important aspect of this research was to investigate the effect of direct pressure on the reaction. Therefore, polymerizations were run in the presence of 1250 psi nitrogen at 50°C for various reaction times. As shown in Figure 37, polyester molecular weights were significantly lower in the nitrogen reactions than both the bulk and carbon dioxide reactions. This was a clear indication that the effect of pressure was a hindrance to the polymerization. In the case of the carbon dioxide reactions, although pressure is producing a negative effect on the reaction, it can be argued that carbon dioxide is decreasing the viscosity of the polymer, thus reducing mass transfer limitations and enhancing molecular weight. Therefore, the effect of pressure is not as pronounced due

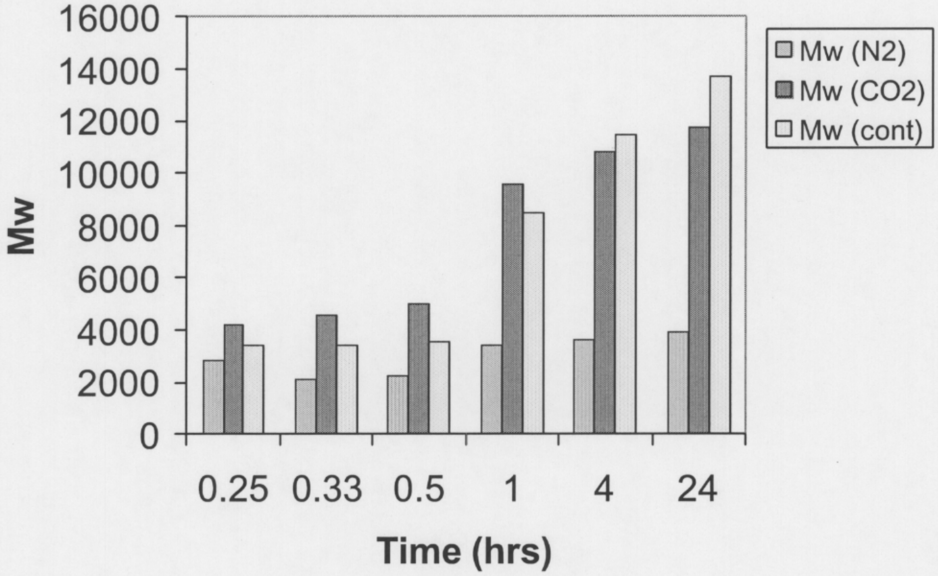


Figure 37 Molecular Weight Comparison of Reactions Run in the Presence of Nitrogen, Carbon Dioxide and a Solvent-Free Environment

to the positive effect of the carbon dioxide. In the case of the nitrogen reactions, there is no such effect to counterbalance the impact of pressure. The results of the nitrogen runs helped to support the hypothesis that the enzyme was being negatively affected by pressure. Thus, it was imperative to examine the stability of the enzyme under these conditions.

9.5 Stability Studies

The stability of Novozym® 435 was examined at 50°C in the presence of divinyl adipate (DVA), 1,4-butanediol (BD), and either 1250 psi carbon dioxide or nitrogen for various times. These results were then compared to stability studies that were performed under bulk conditions at 50°C. As shown in Figure 38, the results of the bulk studies show that after 1 hour, the enzyme still retains over 55% of its original activity. However, when 1250 psi carbon dioxide is present, this value plummets to approximately 16%. The results of the bulk stability studies correlated well to previous reported results for this reaction.(145) These experiments were performed in duplicate in order to ensure the reproducibility of the results.

Once the carbon dioxide studies were completed, stability studies were then conducted under the same conditions, only replacing the carbon dioxide with nitrogen at the same pressure. Figure 39 demonstrates that the results of the nitrogen stability studies were very similar to the carbon dioxide results. These experiments helped to further confirm the hypothesis that pressure has a damaging effect on the enzyme, thus hindering polymer molecular weight after a certain period of time.

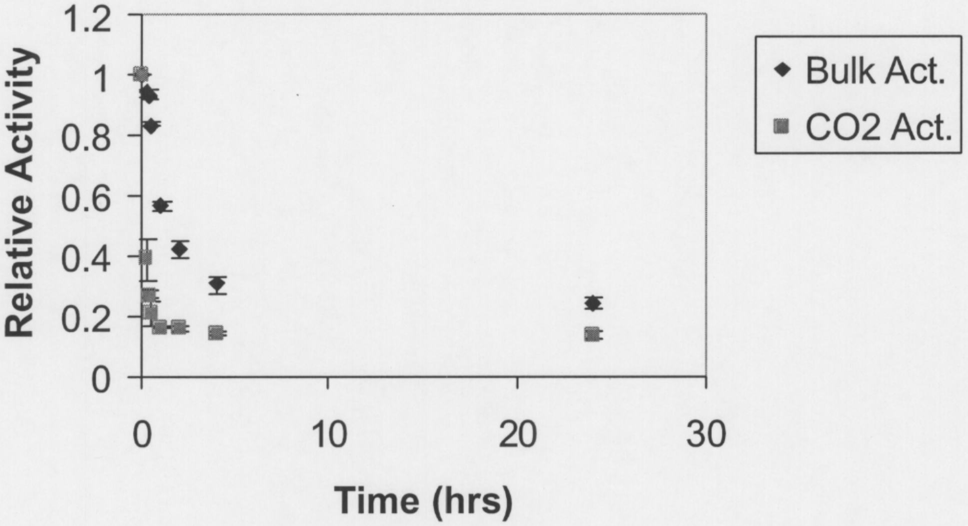


Figure 38 Stability of Novozym® 435 at 50°C in the Presence of Divinyl Adipate and 1,4-Butanediol (Solvent-Free vs. 1250 psi Carbon Dioxide)

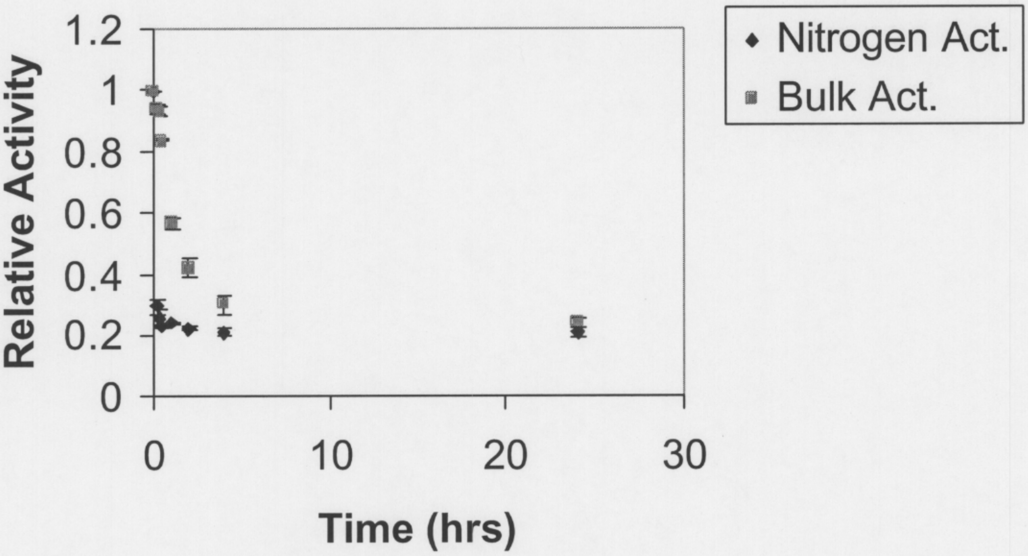


Figure 39 Stability of Novozym® 435 at 50°C in the Presence of Divinyl Adipate and 1,4-Butanediol (Solvent-Free vs. 1250 psi Nitrogen)

After it was confirmed that Novozym® 435 was being adversely affected by pressure, the next step was to ascertain why this was the case. If pressure was somehow limiting the available surface area on a given enzyme bead, this could help to explain the observed results. Therefore, scanning electron microscopy (SEM) and BET analysis were performed on samples of Novozym® 435 that had been exposed to nitrogen and carbon dioxide for given amounts of time in order to examine the particles in greater detail.

9.6 SEM Results of NovozymÒ 435 Beads Exposed to Nitrogen and Carbon Dioxide

Scanning electron microscopy (SEM) was used to examine the morphology of samples of Novozym® 435 beads exposed to carbon dioxide and nitrogen at 1250 psi for increasing lengths of time. These results were compared to a control experiment in which enzyme beads that had not been exposed to pressure were examined using the same method. The electron micrographs of all samples can be seen in Appendix A. A magnification of 10,000 revealed that as time increased, the morphology of the beads dramatically changed. The surface of the bead was no longer uniform, with areas of aggregation increasing with time. Thus, any enzyme that was located in the pores closed off by these domains would no longer be available for catalysis. It is important to remember that these beads on which the enzyme is immobilized to are acrylic resins. Several research groups in the past have been able to show that increased pressure can reduce the porosity of acrylic resins.(146-148)

9.7 BET Analysis of Novozym® 435 Beads Exposed to Nitrogen and Carbon Dioxide

Another way to examine changes in particle morphology is to perform BET analysis on the beads. BET stands for the three men who developed the method: Brunauer, Emmett and Teller. BET measures surface area by the adsorption of gas, usually nitrogen on the solid particle surface. Although several layers of nitrogen molecules can adsorb to the surface, the calculated area corresponds to a monolayer of coverage.(149)

Therefore, BET analysis was used to examine samples of Novozym® 435 that had been exposed to carbon dioxide and nitrogen for increasing lengths of time. Samples of 15 and 60 minute exposures were compared to samples that had not been exposed to pressure in order to determine if there was a decrease in particle surface area. Duplicates of each sample were sent to the testing facility. The results of the BET analysis can be seen in Table 10.

Table 10 BET Surface Area Results

Exposure Time (min.)	N_2 Surface Area (m^2/g)	CO_2 Surface Area (m^2/g)
0	122.364 ± 3.129	122.364 ± 3.129
15	122.024 ± 1.482	120.229 ± 9.197
60	116.513 ± 1.556	119.820 ± 1.267

Although the BET results do not reflect surface area changes of any significance, it is important to remember that BET is a bulk technique. Large sample sizes are required to perform the analysis, thus resulting in data that is reflective of the bulk material. Although duplicates of each sample were sent, Novozym® 435 is composed of beads of varying sizes. In the report sent from Material Testing Laboratory, it was noted that the small, fine particles of the enzyme adhered to the glass vials they were sent in, whereas the larger particles did not. Therefore, when the samples were transferred to the BET sampling tubes, some of the small particles did not transfer, thereby changing the make-up of the sample and the resulting surface area. The bulk surface area of an enzyme sample then depends on the ratio of small to big particles in the vial. This variable could then account for the resulting BET data.

9.8 Conclusion

The solvent-free lipase-catalyzed reaction of divinyl adipate (DVA) and 1,4-butanediol (BD) was studied with and without the presence of supercritical carbon dioxide at 1250 psi and 50°C in order to determine if the carbon dioxide could act as a viscosity-reducing agent. This would then alleviate diffusional limitations and thereby increase polyester molecular weight. It was demonstrated that at reaction times below 1 hour, the presence of supercritical CO_2 did enhance molecular weight. The most pronounced effect was found to be at 30 minutes. However, after 1 hour, this trend began to reverse itself and carbon dioxide appeared to hinder the achievable polymer molecular weight. Therefore, a study was conducted to investigate whether carbon dioxide and/or pressure hindered the reaction. Polymerizations performed in the presence of nitrogen at 1250 psi showed that molecular weight was further limited in this environment. Stability studies confirmed that when the enzyme was exposed for prolonged periods of time to either carbon dioxide or nitrogen, the relative activity dramatically decreased. Scanning electron microscopy experiments supplemented the stability results by showing a change in particle morphology as the time of pressure exposure increased. The results provided by BET analysis proved to be inconclusive due to the nature of the technique.

10.0 SUMMARY AND FUTURE RECOMMENDATIONS

10.1 Summary

Concern for the environment has been a growing sentiment for many years. Although the chemical industry is an integral part of our society and way of life, its traditional processes have a detrimental impact on the environment. Therefore, much research has been dedicated to converting chemical processes to ones that are more environmentally friendly, yet economically viable and feasible. Replacing conventional, corrosive acid catalysts with enzymes eliminates the need for extreme temperatures and pressures since enzymes can function at ambient conditions. Another step in this conversion is substituting organic solvents with benign ones, such as supercritical carbon dioxide and ionic liquids. Supercritical carbon dioxide is nontoxic and highly available and ionic liquids have no measurable vapor pressure.

Initially, supercritical carbon dioxide was intended to be used as a solvent for biocatalytic polymerizations. Conducting an enzymatic reaction in supercritical fluids provides the advantages of solvent tunability, enhanced mass transfer rates, and the ability to control enzyme activity and selectivity. Previous research had shown that polyester molecular weight and polydispersity could be rationally and predictably controlled by using supercritical fluids as the reaction medium.(25) The supercritical fluid used in these studies was fluoroform and it was proposed to replace this fluid with supercritical carbon dioxide. However, polar substrates are poorly soluble in carbon dioxide. Because fluorinated species are known to exhibit higher solubility in CO_2, a study of lipase-catalyzed fluorinated polyester synthesis was undertaken in order to determine if and what molecular weight polymers could be synthesized using fluorinated monomers. Fluorinated polymers are unique due to their interesting properties, such as improved chemical resistance, low water absorption, and low coefficients of friction. After initial experiments indicated that the molecular weight of a fluorinated polyester was significantly lower than that of its hydrogenated counterpart, studies were then performed to ascertain what factors limited chain extension in fluorinated polyester synthesis. After ruling out possibilities such as enzyme inactivation, the equilibrium position for the reaction, polymer precipitation and hydrolysis, it was found that the specificity of the enzyme was the key factor. Novozym®435 was found to favor a fluorinated diol that possessed an optimum combination of chain length and fluorination. The highest molecular weight polyester was obtained by reacting divinyl adipate (DVA) with 3,3,4,4,5,5,6,6-octafluorooctan-1,8-diol (OFOD) using Novozym® 435 as a catalyst at 50°C. Polyester molecular weights averaging 8094 Da (solvent-free) and 3245 Da (THF-based) were achieved.

In conjunction with these experiments, phase behavior studies of DVA, fluorinated diols of varying chain length, and a fluorinated polyester were also conducted in supercritical carbon dioxide. Divinyl adipate was found to be highly soluble, even though it was not fluorinated. With regard to the fluorinated diols, they were more soluble in carbon dioxide than their hydrogenated counterparts. An equimolar mixture of DVA and OFOD also exhibited solubility, as well as a polymer synthesized from DVA and 2,2,3,3-tetrafluoro-1,4-butanediol (TFBD). Once all phase behavior studies were completed, a biocatalytic polymerization between DVA and OFOD was conducted using supercritical carbon dioxide as the solvent. A polyester with molecular weight of 8232 Da was synthesized.

The study of fluorinated polyesters was extended to synthesizing fluorinated-hydroxylated polyesters in the hopes of producing a polymer with interesting properties that could have applications as surfactants or compatibilizers. DVA was reacted with varying ratios of either glycerol or 1,2,6-trihydroxyhexane (HT) and OFOD. Hydroxyl number analysis revealed that when glycerol was used, the hydroxyl numbers were much lower. Therefore, all other experiments were performed with 1,2,6-trihydroxyhexane. As the ratio of OFOD to HT changed, so did the hydroxyl number and molecular weight of the resulting polyester. DVA was also reacted with 1,2,4-butanetriol in order to assess whether or not an optically active polyester could be produced.

Another aspect of this research was to examine enzymatic catalysis in ionic liquids. Ionic liquids are room temperature liquids comprised entirely of ions. Most importantly, they possess no measurable vapor pressure and are thus considered to be environmentally friendly. Stability studies of two enzymes, Novozym® 435 and porcine pancreatic lipase, at 50°C demonstrated that the ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) provided a much more stabilizing environment than tetrahydrofuran. This held true even when the stability studies were conducted in the presence of DVA and BD, at a higher temperature (70°C) and for longer time periods.

Because Novozym® 435 proved to be highly stable in the ionic liquid, polymerization studies were then investigated. DVA was reacted with BD, OFOD, and

HFPD and the resulting polyesters were analyzed via GPC. The highest molecular weight polymer produced was from the reaction between DVA and BD. However, in all cases, the polymerization was limited due to the fact that the growing polymer precipitated out of the ionic liquid.

Supercritical carbon dioxide was also studied in a capacity other than as a reaction medium. Because previous research showed that polyester molecular weight was limited diffusionally by the viscosity of the polymer solution in the molecular weight range of 3,000-5,000 Da,(141) carbon dioxide was employed as a viscosity-reducing agent. Reactions between DVA and BD were carried out at 50°C using Novozym® 435 with and without the presence of supercritical carbon dioxide (1250 psi). It was found that while carbon dioxide enhanced polyester molecular weight for reaction times below 1 hour, the opposite effect occurred at times longer than 1 hour. Reactions with nitrogen indicated that the polymerization was hindered by pressure in some way. Stability studies further confirmed that when the enzyme was exposed to pressure for 24 hours, its relative activity significantly decreased. Scanning electron microscopy experiments revealed that the morphology of the enzyme particles changed with increased time of exposure to pressure.

10.2 Future Recommendations

Although much progress was made with the original research objectives, certain aspects of this project can be continued. Even though fluorinated-hydroxylated polyesters were synthesized and characterized, it was never determined what their applicability could be. Therefore, large quantities of each type of polymer could be made in order to perform physical property tests on the materials to investigate what they could possibly be used for.

Supercritical carbon dioxide has been shown to enhance polyester molecular weight for reaction times below 1 hour. However, because this trend reverses itself after that time, studies should be conducted in order to address this issue. Other enzymes should be examined for their sensitivity to pressure. Previous studies using supercritical fluids used lyophilized porcine pancreatic lipase (PPL) as the catalyst. Therefore, further studies could be conducted using this enzyme. Also, viscosity measurements should be taken for polymer samples that were exposed to carbon dioxide and compared to ones that were not in order to ascertain if CO_2 did indeed reduce the viscosity. It could also be investigated as to whether or not CO_2-philic species, such as fluorinated compounds would enhance any observed effects.

As far as the ionic liquids aspect of this project is concerned, research has just recently begun on several new ionic liquids comprised of various cations and anions (Table 11). It should be noted that all but one of these new ionic liquids are hydrophilic, as opposed to the previous one used for the experiments.

Table 11 Additional Ionic Liquids to be Tested as Potential Solvents

	Name	Formula
1	N-methyl-N-(2-methoxyethyl) pyrrolidinium hexafluorophosphate	$(C_8H_{18}NO)^+ (PF_6)^-$
2	1-butyl-3-methylimidazolium acetate	$(C_8H_{15}N_2)^+ (CH_3CO_2)^-$
3	1-butyl-3-methylimidazolium nitrate	$(C_8H_{15}N_2)^+ (NO_3)^-$
4	1-butyl-3-methylimidazolium trifluoroacetate	$(C_8H_{15}N_2)^+ (CF_3CO_2)^-$
5	N-methyl-N-(2-methoxyethyl) pyrrolidinium methanesulfonate	$(C_8H_{18}NO)^+ (CH_3SO_3)^-$
6	N-methyl-N-(2-methoxyethyl) pyrrolidinium acetate	$(C_8H_{18}NO)^+ (CH_3CO_2)^-$
7	N-methyl-N-(2-methoxyethyl) pyrrolidinium nitrate	$(C_8H_{18}NO)^+ (NO_3)^-$
8	N-methyl-N-(2-methoxyethyl) pyrrolidinium trifluoroacetate	$(C_8H_{18}NO)^+ (CF_3CO_2)^-$
9	N-methyl-N-(2-methoxyethyl) pyrrolidinium trifluoromethanesulfonate	$(C_8H_{18}NO)^+ (CF_3SO_3)^-$

The solubility of DVA and BD, as well as other monomers of interest, such as fluorinated and hydroxylated should be tested in each of the above mentioned ionic liquids to assess whether or not polymerizations could be performed in each of them. Then, if the growing polymer does not proceed to precipitate out of the solution, as in previous studies, it must be determined how to separate the polymer from the ionic liquid, since they have no vapor pressure. One possibility could be extraction with a benign solvent. Additionally, enzyme activity and stability should be measured in each of the ionic liquids, as well as a comparison of reaction rates in ionic liquids versus conventional organic solvents, such as hexane. In addition to these potential solvent replacements, the ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate could also be synthesized in our laboratory and examined for its ability to support enzyme activity and stability, as well as a solvent for biocatalytic polymerizations.

The rest of the work to be accomplished in this area of research should be focused on fulfilling the required objectives on the EPA grant. Four aims were included in the grant:

1) Test the general hypothesis that mass transfer is responsible for limiting the molecular weight of the polymer is solvent-free biocatalytic polymerization. In examining this hypothesis, we will investigate the roles of both internal and external diffusion by varying: a) catalyst porosity, b) catalyst support, c) enzyme particle size, d) reactor configurations, e) agitation speed, and f) viscosity. We speculate that pore diffusion only contributes to mass transfer resistance up to the critical molecular weight of the polymer.

2) Demonstrate that a reduction in viscosity through the use of liquid or supercritical CO_2 can reduce the external mass transfer limitations during the polymerization. Further, because CO_2 is used only as a viscosity modifier, full solubility of substrates is not necessary. To investigate the importance of compatibility of the monomers with CO_2, we will measure the impact of using fluorinated components in the monomers.

3) From the information acquired after studying the above two aims, we will focus on the molecular weight optimization of polymers that are difficult to synthesize using chemical means, such as polyesters with pendant hydroxyl groups. The use of

enzymes will allow us to capitalize on their inherent specificity to accomplish the synthesis. Once we have developed an understanding of the breadth of enzyme specificity in biocatalytic polytransesterification, we will initiate a search for ester substrates which are productive without releasing acetaldehyde as a byproduct.

4) Selecting a series of model polymerizations, and using the knowledge generated from aims 1-3 to guide our studies, we will perform biocatalytic polyesterifications in ionic liquids. We will study the stability, activity and specificity of the enzymes in these novel solvents, and we will attempt to develop structure-function-environment relationships for systems which demonstrate significant activity.

Although many of these aims have been addressed previously, the following work is still in the future. For Aim #1, the variation of catalyst porosity still needs to be investigated. The proposed plan involves purchasing acrylic resins of varying pore sizes and immobilizing Novozym® 435 to them. If it is then discovered that the polymerizations are indeed limited by internal diffusion, then the use of dissolved enzyme preparations will be studied.

The first step in continuing Aim #3 has already been accomplished in our laboratory by synthesizing diisopropenyl adipate. This monomer is considered to be an attractive alternative to divinyl adipate because when it is polymerized with 1,4-butanediol, the by-product of the reaction is acetone, as opposed to acetaldehyde. Research has indicated that a polymer can be formed using this new monomer, however molecular weights were not as high as with divinyl adipate. Therefore, studies should be directed at trying to optimize this reaction and improve polymer molecular weight.

Suggestions for Aims #2 and #4 have been addressed previously in this section. Once this work is completed, all goals stated on the grant will have been achieved and will hopefully indicate that much progress has been made towards creating processes that are more environmentally friendly, yet practical and with wide applicability. Biocatalysis holds tremendous promise for the future due to the fact that it is environmentally benign and requires low energy utilization and waste generation. The research conducted in our laboratory has helped to further the goal of implementing biocatalysis into everyday chemical technology.

APPENDIX A

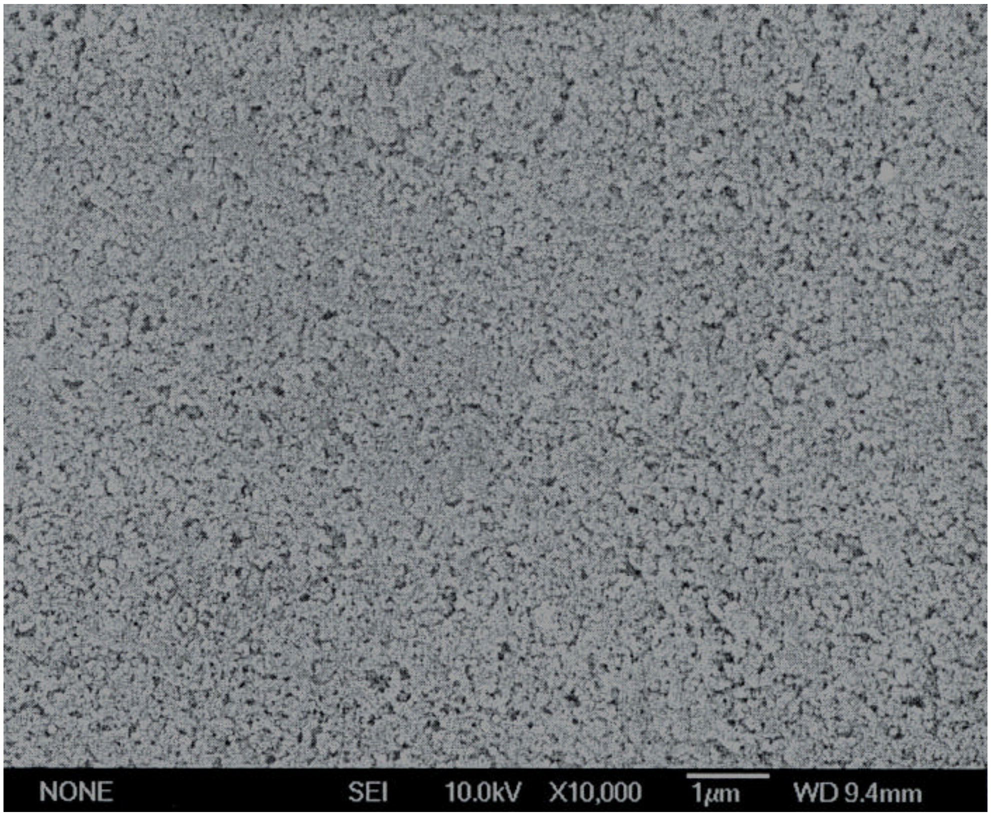


Figure A1 SEM Photograph of Novozym® 435 (Control)

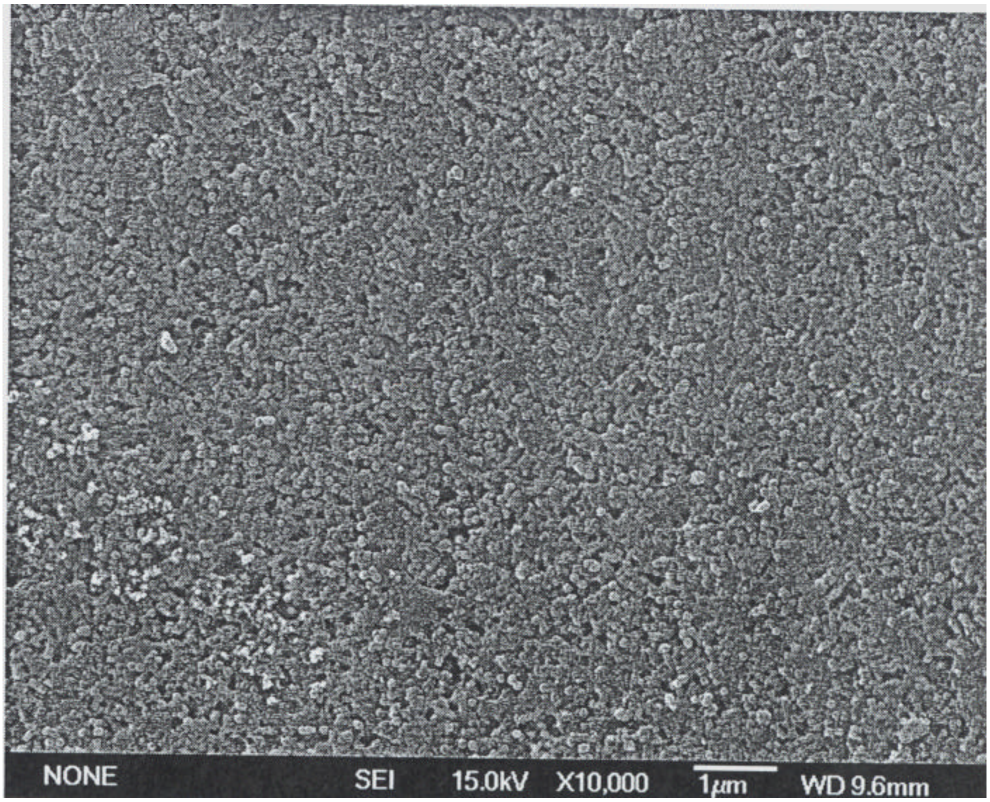


Figure A2 SEM Photograph of Novozym® 435 Exposed to Carbon Dioxide for 1 Minute

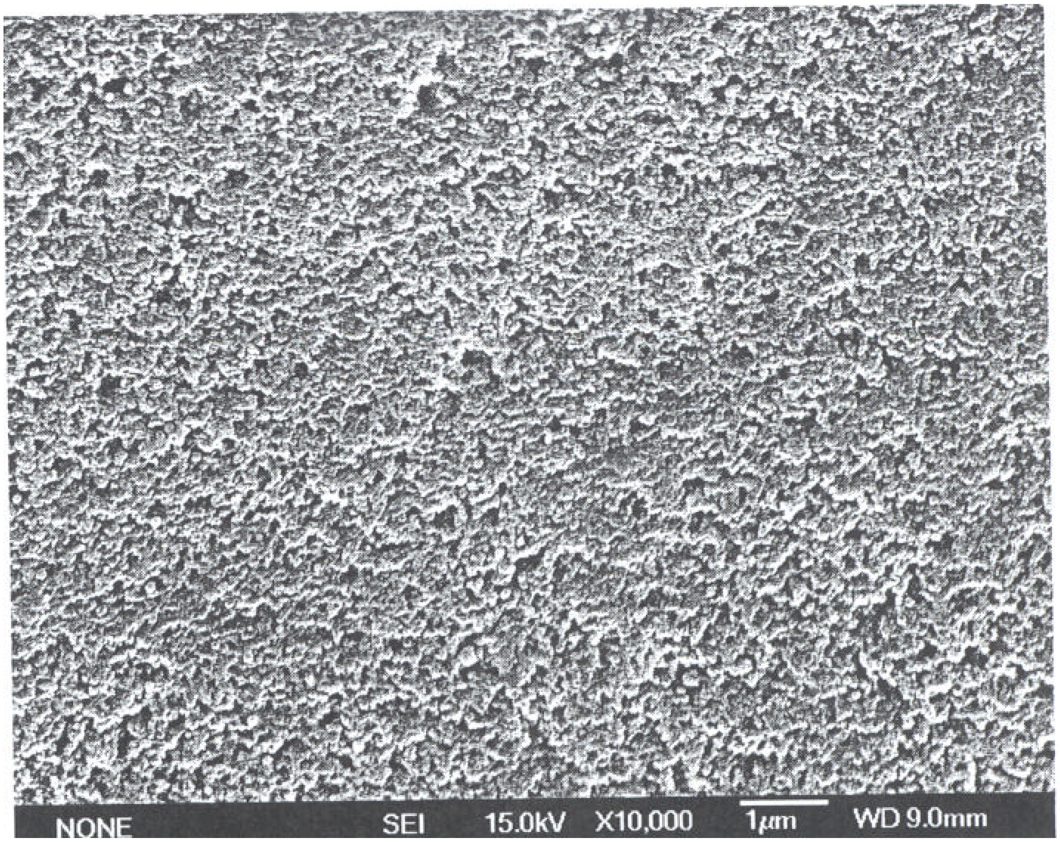


Figure A3 SEM Photograph of Novozym® 435 Exposed to Carbon Dioxide for 2 Minutes

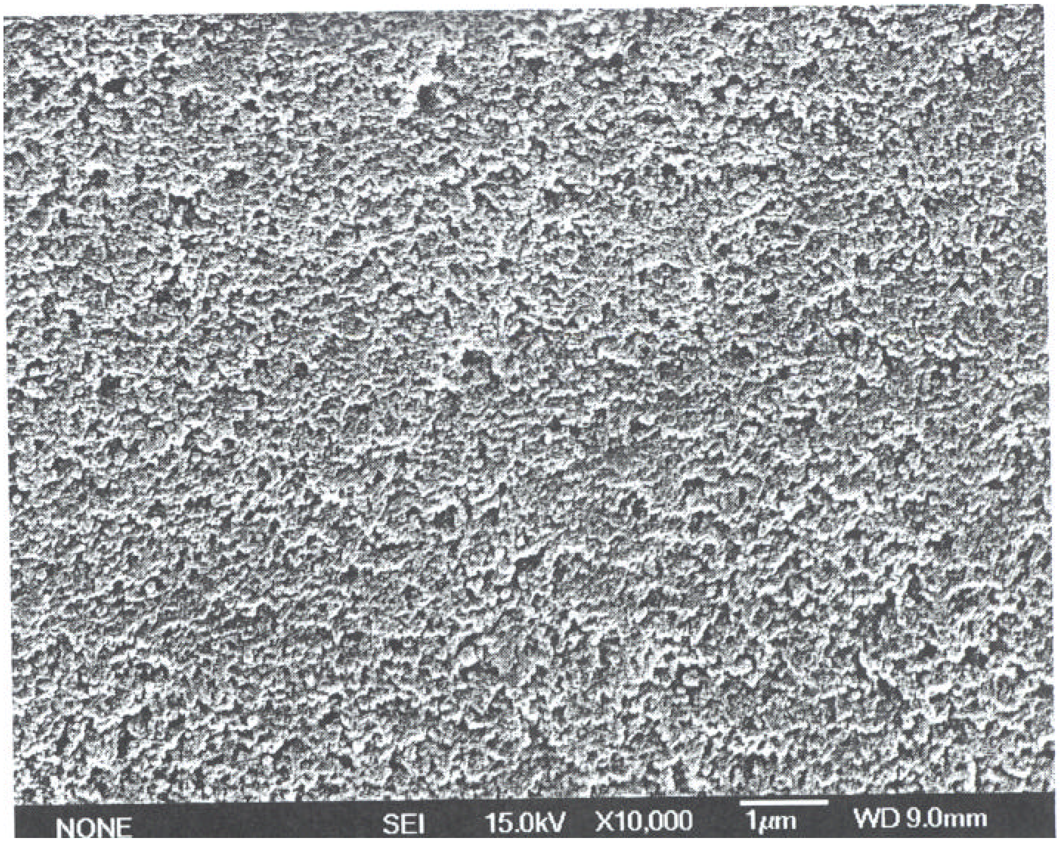


Figure A4 SEM Photograph of Novozym® 435 Exposed to Carbon Dioxide for 5 Minutes

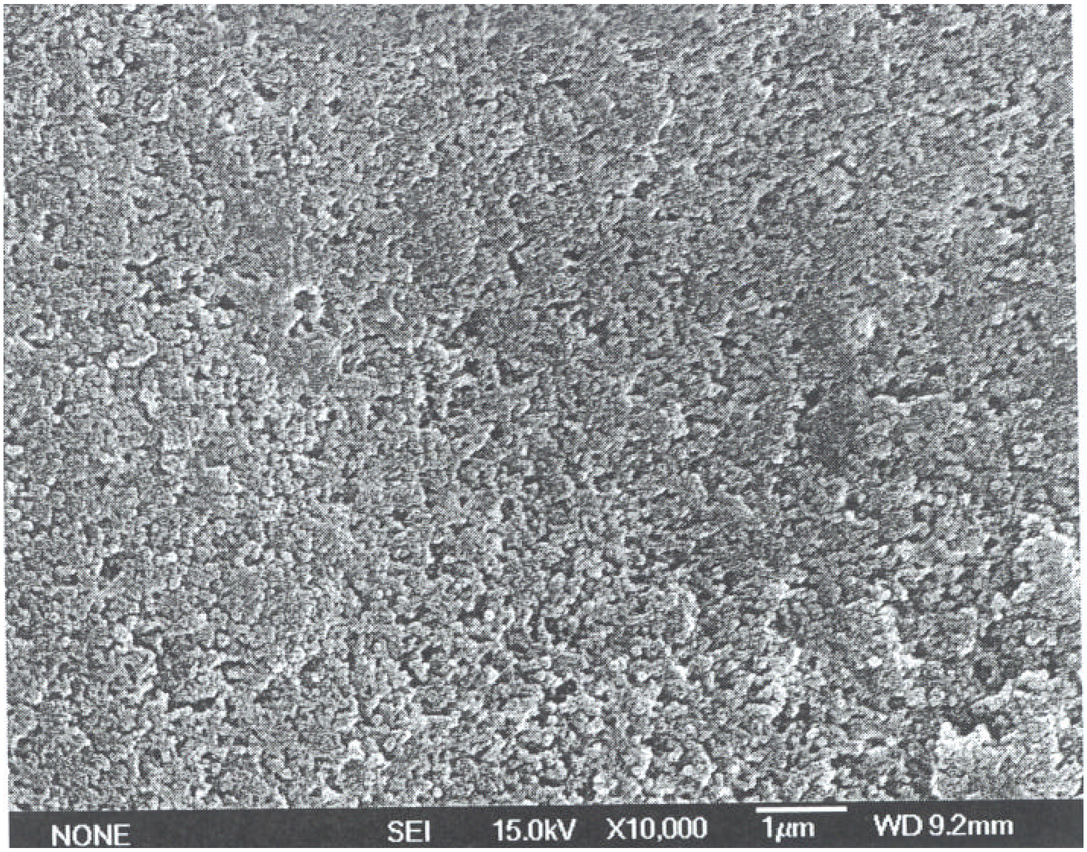


Figure A5 SEM Photograph of Novozym® 435 Exposed to Carbon Dioxide for 20 Minutes

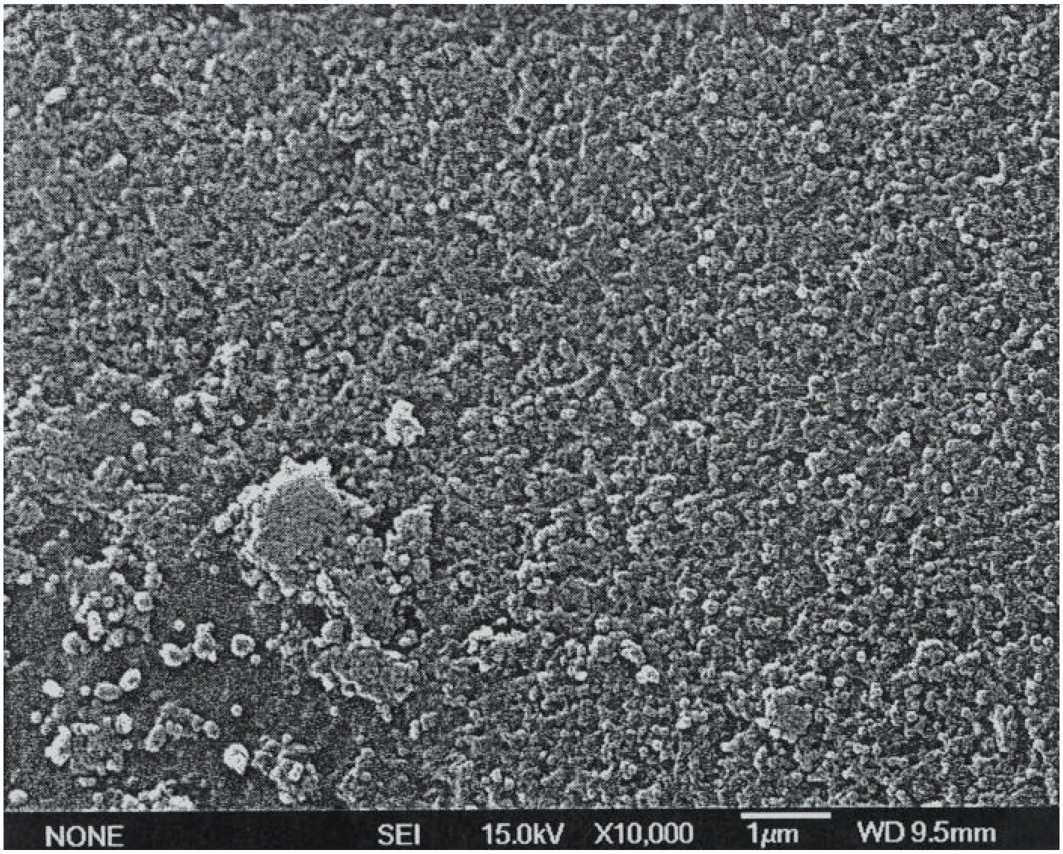


Figure A6 SEM Photograph of Novozym® 435 Exposed to Carbon Dioxide for 60 Minutes

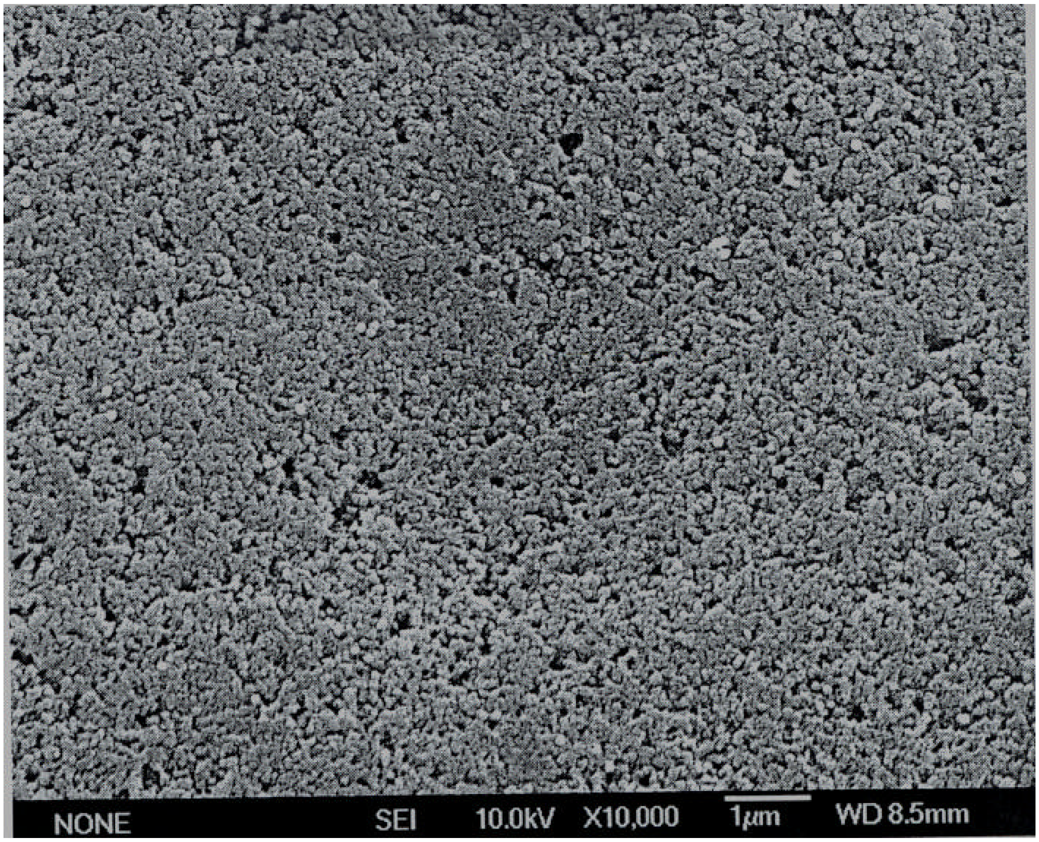


Figure A7 SEM Photograph of Novozym® 435 Exposed to Nitrogen for 1 Minute

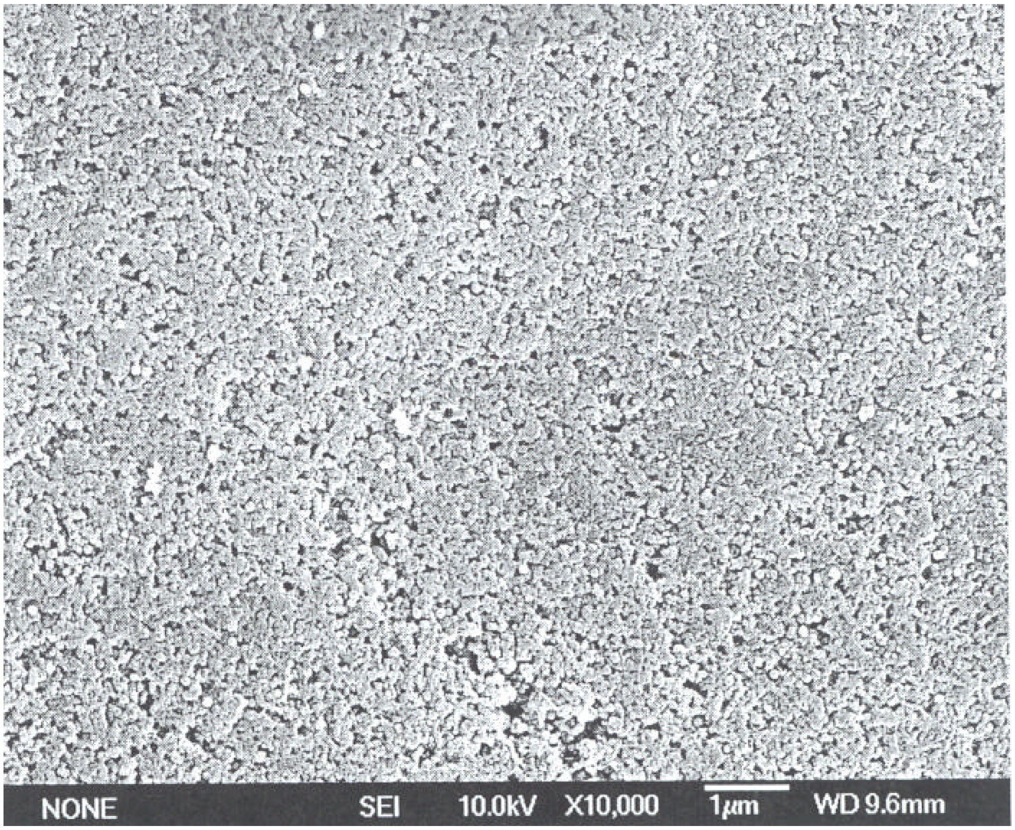


Figure A8 SEM Photograph of Novozym® 435 Exposed to Nitrogen for 2 Minutes

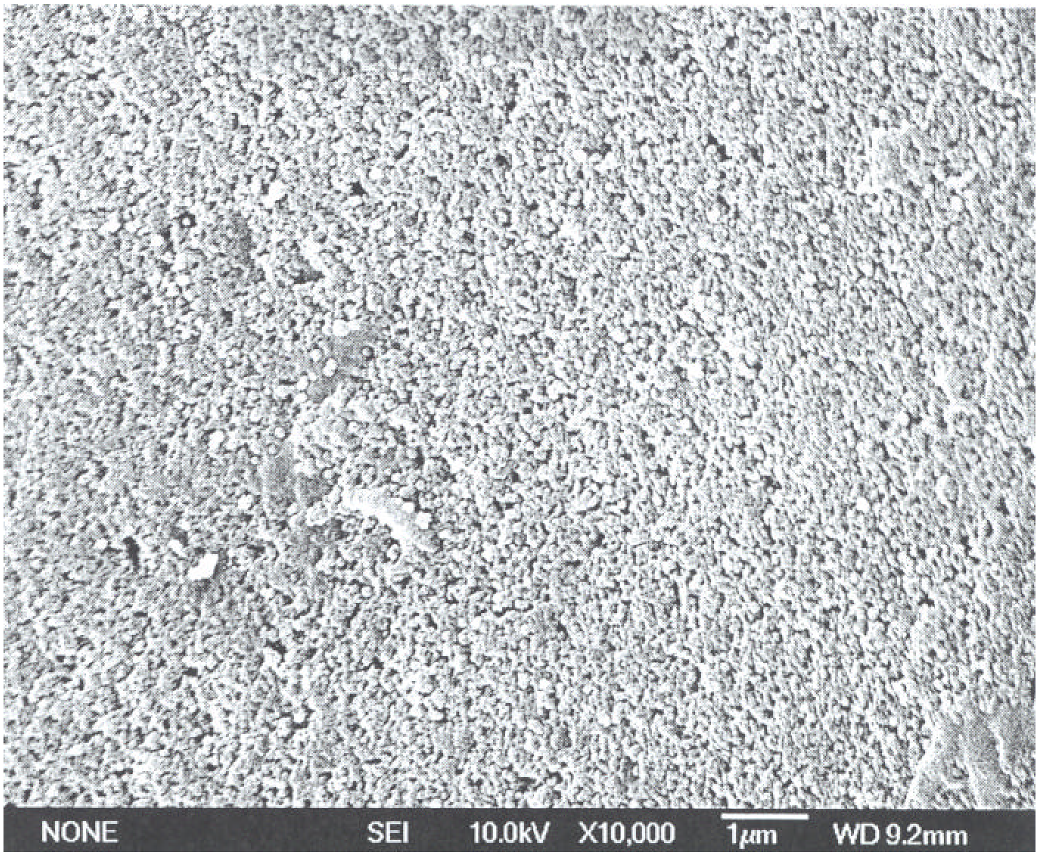


Figure A9 SEM Photograph of Novozym® 435 Exposed to Nitrogen for 5 Minutes

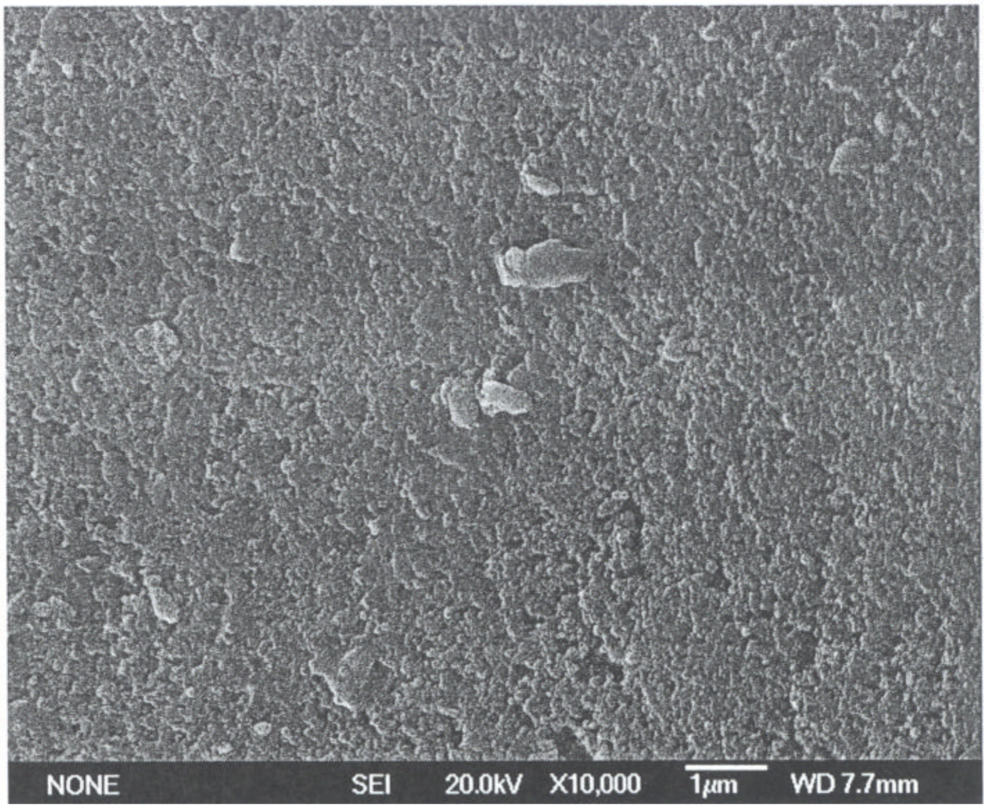


Figure A10 SEM Photograph of Novozym® 435 Exposed to Nitrogen for 20 Minutes

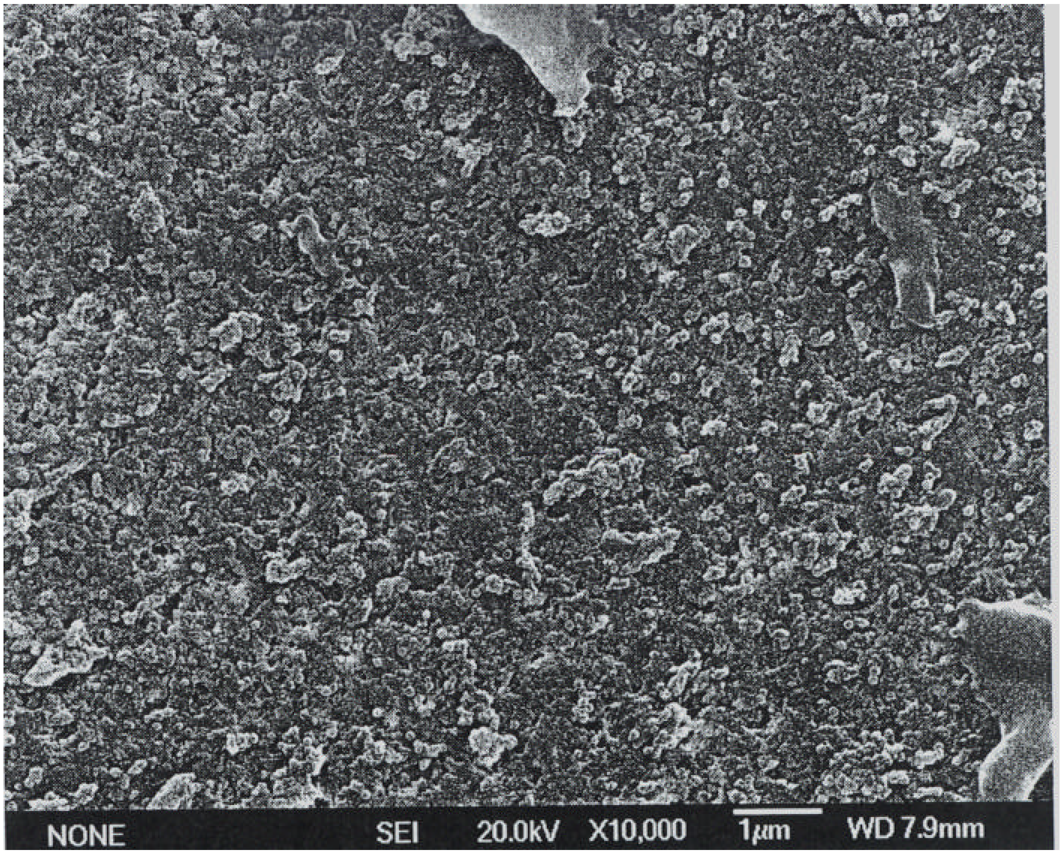


Figure A11 SEM Photograph of Novozym® 435 Exposed to Nitrogen for 60 Minutes

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